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Characterisation of immune cell function in fragment and full-length Huntington's disease mouse models

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ABSTRACT

Inflammation is a growing area of research in neurodegeneration. In Huntington's disease (HD), a fatal inherited neurodegenerative disease caused by a CAG-repeat expansion in the gene encoding huntingtin, patients have increased plasma levels of inflammatory cytokines and circulating monocytes that are hyper-responsive to immune stimuli. Several mouse models of HD also show elevated plasma levels of inflammatory cytokines. To further determine the degree to which these models recapitulate observations in HD patients, we evaluated various myeloid cell populations from different HD mouse models to determine whether they are similarly hyper-responsive, as well as measuring other aspects of myeloid cell function. Myeloid cells from each of the three mouse models studied, R6/2, *Hdh*Q150 knock-in and YAC128, showed increased cytokine production when stimulated. However, bone marrow CD11b^{+} cells did not show the same hyper-responsive phenotype as spleen and blood cells. Furthermore, macrophages isolated from R6/2 mice show increased levels of phagocytosis, similar to findings in HD patients. Taken together, these results show significant promise for these mouse models to be used to study targeting innate immune pathways identified in human cells, thereby helping to understand the role the peripheral immune system plays in HD progression.

Huntington's disease (HD) is an autosomal dominant inherited neurodegenerative disease caused by a CAG-expansion in exon 1 of the huntingtin (*HTT*) gene, resulting in a mutant form of the protein (mHTT) that contains an elongated polyglutamine tract (The Huntington's Disease Collaborative Research Group, 1993). It is characterised by motor, cognitive and neuropsychiatric symptoms, which are associated with a loss of medium spiny neurons in the striatum and other changes in the CNS (Vonsattel and DiFiglia, 1998). However, patients are also affected by peripheral symptoms such as weight loss, muscle wasting and inflammation (van der Burg et al., 2009).

Animal models are a vital tool to research genetic diseases. In HD, many animal models have been established, including invertebrate models such as *Drosophila melanogaster* (Kazemi-Esfarjani and Benzer, 2000) and *Caenorhabditis elegans* (Parker et al., 2001), and mammalian models from rodents (Crook and Houseman, 2011) to primates (Yang et al., 2008). Invertebrate models have great potential for drug discovery studies such as therapeutic screens due to their short life-span and cost efficiency (Voisine et al., 2007), but they may not always be useful due to their relative lack of homology to the human genome. For example, invertebrate immune responses are very different to those in humans, with an innate immune system that shows distinct differences and the complete absence of an adaptive immune system (Beck and Habicht, 1996).

A variety of HD mouse models have been developed. The most widely used and best characterised is the R6/2, which ubiquitously expresses the 5′ end of the human *HTT* gene carrying only exon 1 with 150 CAG repeats (Mangiarini et al., 1996). The mice demonstrate a fast and progressive phenotype with a very early symptomatic onset at 6–8 weeks, showing motor symptoms, loss of brain volume and peripheral changes such as weight loss (Bjorkqvist et al., 2006; Li et al., 2005; Mangiarini et al., 1996). Moreover, these mice are a model of the mis-splicing of the *HTT* gene that occurs to generate an exon 1 HTT protein in all full length HD mouse models (Sathasivam et al., 2013).

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Transgenic and knock-in mouse models expressing full-length mHTT have also been developed. The *Hdh*Q150 model was generated by knocking-in an expanded repeat of 150 CAGs into the mouse *Htt* gene (Lin et al., 2001) and develops progressive HD related phenotypes until end-stage disease at approximately 22 months of age (Woodman et al., 2007). At late-stage disease, the R6/2 mice (12–14 weeks) are remarkably comparable to *Hdh*Q150 mice (22 months), with both models exhibiting weight loss, motor abnormalities and widespread mHTT aggregates throughout the brain (Woodman et al., 2007), aggregates in peripheral tissues (Moffitt et al., 2009), highly comparable transcriptional profiles (Kuhn et al., 2007) and a progressively impaired heat shock response (Labbadia et al., 2011). YAC128 mice are transgenic for the full-length human *HTT* gene originally containing 128 CAG repeats (Slow et al., 2003). They develop progressive motor deficits from the age of six months, and show selective cortical and striatal atrophy at nine months (Van Raamsdonk et al., 2005).

HD patients have elevated plasma levels of inflammatory cytokines and chemokines (Bjorkqvist et al., 2008; Wild et al., 2011), and their monocytes are hyper-reactive following lipopolysaccharides (LPS) stimulation in vitro (Träger et al., 2014). In mice, the R6/2, *Hdh*Q150 and YAC128 models each have similarly elevated plasma levels of inflammatory cytokines (Bjorkqvist et al., 2008). Myeloid cells from both R6/2 and BACHD mice have also been shown to migrate abnormally (Kwan et al., 2012b), demonstrating that they are affected by mHTT expression. Here, myeloid cells from different HD mouse models were evaluated to assess whether they are similarly hyper-responsive, like HD patient cells. We analysed cytokine production and other key immune cell functions in cultured cells, comparing several commonly-studied myeloid cell populations from blood, spleen and bone marrow.

Materials and methods

Animals

Mice from colonies at King's College London (KCL) were used for the cytokine profiling, phagocytosis assays and FACS analyses. R6/2 mice (Mangiarini et al., 1996) were bred by backcrossing hemizygous males to (CBA × C57BL/6)-F1 females (B6CBAF1/OlaHsd; Harlan, UK). *Hdh*Q150 homozygous mice on a (CBA × C57BL/6) F1 background ($Hdh^{150Q/150Q}$) were obtained by inter-crossing *Hdh*Q150 heterozygous CBA/Ca and C57BL/6J congenic lines as described previously (Woodman et al., 2007). For the differentiation and cell adhesion assays, R6/2 mice from a colony at Lund University were used (Jackson Laboratories, USA), obtained by crossing heterozygous (C57BL/6) males with (C57BL/6) females. Experiments using YAC128 mice were undertaken at the University of British Columbia, Vancouver. All animals had unlimited access to food and water, were subject to a 12-h light/dark cycle and housing conditions and environmental enrichment were as previously described (Hockly et al., 2003).

At KCL, R6/2 and *Hdh*Q150 mice were genotyped by PCR using genomic DNA isolated from an ear-punch and the *Htt* CAG repeat length was measured as previously described (Sathasivam et al., 2010). The CAG repeat size for the KCL R6/2 mice was 209.3 ± 8.5 and for the *Hdh*Q150 mice it was 163.3 ± 2.8. At Lund University, tail tips were taken at 4 weeks of age and genotyped by PCR at Laragen (Laragen, USA). The CAG repeat length size for the Lund R6/2 mice was 240–252 repeats. The YAC128 mice had a CAG repeat length of 120 ± 0.343.

At KCL, all experimental procedures were approved by the Local Ethics Committee and performed under a project licence issued by the UK Home Office. Experimental procedures at Lund University were carried out in strict accordance with Swedish legislation and approved by the Animal Ethics Committee in Lund and Malmö, Sweden, and at the University of British Columbia they were performed in accordance with protocols approved by the University of British Columbia Committee on Animal Care and the Canadian Council on Animal Care.

Isolation of primary murine cells

Blood monocytes

Blood was obtained by cardiac puncture, collected into EDTA coated tubes (BD Vacutainer), and 1 ml red blood cell lysis buffer (ammonium chloride buffer; eBioscience) was added per 1 ml of blood. The samples were then incubated for 5 min at room temperature. Following centrifugation at 300 ×*g* for 5 min, the lysis step was repeated twice. Cells were then resuspended in 270 μl MACS buffer (PBS including 1% bovine serum albumin (BSA) and 2 mM EDTA) and 30 μl anti-mouse CD11b magnetic beads. After 15 min incubation in the fridge, the samples were washed in MACS buffer (300 ×*g* for 5 min), resuspended in 500 μl MACS buffer and loaded on pre-wetted MACS columns placed in the magnet. After allowing the cell suspension to flow through by gravity, the columns were washed three times with 1 ml MACS buffer. Labelled $CD11b^+$ monocytes were eluted by removing the columns from the magnetic field.

Bone marrow

Mice were sacrificed by neck dislocation or by rising concentration of CO_2. Femur and tibia were dissected at the hip joint and any remaining muscle tissue was carefully removed. The bones were placed in a petri dish filled with cold RPMI-1640 media and cut at the joints. Bone marrow was flushed out by rinsing the shaft with media using a 5 ml syringe and 26 gauge needle. Lumps of cells were disaggregated by pipetting up and down several times before the cells were passed through a 70 μm nylon cell strainer. After washing with RPMI-1640 media (centrifugation at 300 ×*g* for 5 min) cells were counted using a Neubauer counting chamber. The cell suspension was labelled with 10 μl anti-mouse CD11b magnetic beads and 90 μl MACS buffer per 1×10^7 cells, and sorted as described above. When seeded in culture the isolated CD11b positive cell population resembled an early monocyte population, which could then be differentiated into bone marrow-derived macrophages. For the differentiation, sorted bone marrow cells were cultured in R10 media (RPMI-1640 supplemented with 10% FBS, 2 mM L-glutamine, 50 units/ml penicillin and 50 mg/ml streptomycin supplemented with 20 ng/ml recombinant murine M-CSF). After 3 days cells were provided with fresh media and growth factor. The cells resembled a macrophage phenotype from day 6.

Spleen

Spleens were dissected from the mice and stored in RPMI-1640 media until the preparation was started. To obtain a single cell suspension, spleens were cut into pieces and digested using 2 ml digestion buffer (RPMI-1640 media including 10% FBS, 15 mM HEPES, 0.5% collagenase type 4 (C1889; Sigma-Aldrich)). After 30 min incubation at room temperature the remaining spleen pieces were filtered through a 70 μm nylon cell strainer (BD Falcon). Afterwards, the splenocytes were centrifuged (300 ×*g* for 5 min) and the pellet was incubated with 1 ml red blood cell lysis buffer for 5 min at room temperature. Cells were then counted using a Neubauer counting chamber and isolated using 10 μl anti-mouse CD11b magnetic beads and 90 μl MACS buffer per 1×10^7 cells as described for blood monocytes.

Peritoneal macrophages

In order to prevent bleeding into the abdominal cavity, mice were killed using a rising concentration of CO_2. Fur covering the peritoneum was carefully removed and 5 ml of ice-cold, serum free RPMI1640 medium was injected into the abdominal cavity using a 26 gauge needle. After massaging the mouse abdomen for 1–2 min, cells were recovered through a small incision using a sterile plastic Pasteur pipette and put on ice as quickly as possible. The centrifuged (5 min at 300 ×*g*) cell suspension was incubated with 1 ml red blood cell lysis for 5 min at room temperature. Afterwards, the cells were washed with PBS (centrifuged at 300 ×*g* for 5 min). The resultant cell suspension contained macrophages as well as neutrophil, so cultures were enriched for