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A mouse model of HIV associated neurocognitive disorders: a brain-behavior approach to discover disease mechanisms and novel treatments

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The manifestations of HIV infection of the brain, both pathological and clinical, fundamentally changed in the 1990's when combined antiretroviral therapy (cART) became available [1]. Consequently, the incidence of HIV associated dementia (HAD) significantly declined, and milder forms of HIV associated cognitive dysfunction became commonly recognized. In 2007, a group of clinical experts formulated terminology for HIV associated neurocognitive disorders (HAND), which is now widely accepted [2]. Three HAND categories were defined: HAD, and two mild forms of HAND, asymptomatic neurocognitive impairment (ANI) and mild neurocognitive disorder (MND). ANI and MND, which are by definition mild and require special neuropsychological testing for diagnosis, are by far the most prevalent forms of HAND in countries that have access to cART. It is also important to note that despite cART, HAND occurs in approximately 50% of HIV-infected individuals, and it appears to be increasing as cART patients reach old age [3]. In this aging population of cART HIV-infected individuals, there are co-morbidities that frequently develop including neurodegenerative diseases like Alzheimer's disease, stroke and cardiovascular disease, and traumatic brain injury, among others. Therefore, the existence of ANI and MND as independent manifestations of HAND in the absence of co-morbidities has been somewhat controversial. Nevertheless, most NeuroAIDS experts believe that ANI and MND are primarily due to the persistence of HIV in the brain, and that these milder forms of HAND will eventually lead to HAD [4]. Hence, given the prevalence of HAND even with cART, there is a great need to develop novel therapies that either address and effectively treat the mechanisms of cognitive dysfunction in HAND to attenuate symptomology, or that eradicate HIV from the CNS.

Animal models that portray fundamental features of human disease are critical to the investigation and discovery of disease pathogenesis, as well as to the development of innovative therapies [5]. Indeed, animal models allow evaluation of questions in a systematic manner since viral and other relevant exposures can be methodically manipulated and administered, while controlling for or obviating issues that can impact, and interact with, outcomes that can cloud interpretations in human studies. Relevant factors that can be controlled or obviated using animal models include environmental conditions and exposures,

diet, genetic variations, and socio-economic-status. The focus of this article will be on features of HAND that are most important, and that can be modeled in the framework of current scientific technology and knowledge. Context will be provided regarding how the mouse model of HAND fits into current clinical and pathological literature.

Of note, there are several HAND models in use; they have significantly contributed to our understanding of the disease process and have helped to create a landscape in which novel treatments can be discovered [6–9]. This field is growing, and while it is exciting, it is also markedly complex; in this context, it is beyond the scope of this article to compare and contrast current animal models for HAND or HIV encephalitis (HIVE), its supposed histopathological counterpart.

The data presented above support the assertion that an important feature of a HAND animal model is the ability to demonstrate behavioral abnormalities that are similar to the cognitive dysfunction seen in humans. It can be argued that animal models that feature mild learning and/or memory disturbances would best reflect the mild neurocognitive dysfunction seen in ANI and MND. The types of learning or memory deficits seen in humans with HAND that can be modeled in animals are also likely important. For example, visual episodic memory is impaired in HIV-infected individuals [10], and similar functions can be monitored in mice (see mouse HAND model testing below). In addition, understanding the early pathogenesis of mild HAND forms could lead to the development of novel therapeutics based on these discovered mechanisms of disease. ANI and MND probably represent physiological, reversible dysfunction of neurons, whereas HAD likely represents neuronal death and irreversible neurocognitive dysfunction to varying degrees. Innovative treatments would then typically be tested in humans, with the overarching goal to reverse cognitive dysfunction and prevent the advancement from mild HAND to HAD.

It is likely that the classic pathology of HIVE largely reflects the pathological substrate of HAD; HIVE pathology has decreased since the advent of cART, and this has been accompanied by the decrease in HAD incidence [11]. This makes sense because individuals with ANI and MND are less likely to die and undergo detailed brain autopsy evaluations. Thus, autopsy-based studies are dominated by end stage disease or terminal events that result in severe cognitive dysfunction (i.e., dementia). It follows that particularly since the advent of cART, the specific neuropathological correlates of ANI and MND are even less clear than histopathological correlates of HAD in the cART era. It can consequently be argued that an animal model of mild HAND (ANI and MND) that relies solely on neuropathological parameters without accompanying abnormal behavioral evaluations, is not an optimal model. This is a particularly salient point from the standpoint of novel drug development. Indeed, the tenet driving a drug discovery-motivated preclinical scientific effort and program of research is to appropriately model early/mild HAND when it is treatable, not HAD when neuronal death is substantially more prominent and the condition is more likely to be irreversible. This essentially comes down to a framework of effective treatment when the CNS has the plasticity to respond and correct, versus treatment attempts to reverse extreme damage wherein the window of plasticity to respond and correct has likely narrowed significantly or even closed. In the former scenario, rescue and salvage are likely; in the latter scenario, they are not.

Early on in the development of the model to be described, it was recognized that the original histopathological report of HIV in severe combined immunodeficient (SCID) mice needed a behavioral component [12]. These mice are injected intracranially (IC) into the right frontal lobe with 100,000 HIV-infected or uninfected (controls) human monocyte derived macrophages (MDM). Using this model, these human MDM generally are demonstrated to be present from the frontal cortex into the basal ganglia, and the xenografts gradually disappear over a period of approximately 1 month. *There is a gradient of HIV-infected human MDM with most being present around the injection site and less peripherally from the injection site.* These HIV mice, when observed in their cages, appear normal. Subsequently, these HIV mice were shown with Morris water maze testing to exhibit cognitive deficits [13]. Later, it was shown that water radial-arm maze (WRAM) testing could differentiate relatively subtle differences in abnormal behavior of HAND mice due to a single amino acid substitution in the tat gene [14, 15]. The Morris water maze and the WRAM tests largely reflect hippocampal based learning and memory, which, importantly, are also the types of cognitive deficits seen in humans with mild HAND [10]. The neuropathology of SCID mice with HAND includes increased numbers of mononuclear phagocytes and astrocytes, and decreases in the neuronal marker MAP2 immunostaining [13, 16–18]. These histopathological features correlate with behavioral deficits, and cART and other treatments given to SCID mice with HIV can result in improvements of these histopathological features [18–22]. These findings underscore the importance of studying disease models from a systems approach and evaluating brain-behavior relationships, in order to truly ask and answer translational questions.

These studies initially testing drug treatment in HAND mice initiated therapies that temporally occurred simultaneously with the IC injection of HIV-infected- or uninfected-human MDM [18–22]. Although these treatments were usually effective in preventing the behavioral deficits shown by Morris water maze or WRAM testing, they did not show reversibility with the treatment. Showing reversibility with treatment is critical for moving novel treatments to humans with HAND. Indeed, treatments for HAND would typically be instituted only after the condition is diagnosed. Therefore, our efforts over the last several years have been focused on adapting a behavioral paradigm that would allow testing before and after treatments.

The object recognition test (ORT) evaluates the animal's ability to familiarize itself with a set of objects, and then in a subsequent test, differentiate between a previously presented object and a new object. If an animal has successful object memory retention, the animal will preferentially attend to the new object; this can be quantified [23, 24]. The ORT is represented in Figure 1. The initial testing evaluates animals after IC injection of human MDM, but before any drug treatment is introduced (Figure 2). On Day 4 after IC injection of human MDM, the mice experience 10 minutes of acclimation to a Plexiglas chamber (60 cm×60 cm). The next day, on Day 5, the ORT is comprised of a training phase, followed by a 5 minute delay, and then an object preference test. In the training phase, duplicate copies of an object are placed near the two corners at either end of opposite sides of the arena (15 cm from each adjacent wall). The animal is placed into the arena and allowed 5 minutes of exploration of objects (A1) and (A2). During preference/novel object testing (5 minute duration), the animal is replaced in the arena, presented with two objects in the same spatial

positions as they were for training: one object (A3) is a third identical copy of the set of the objects used in the training phase, and the other object is a novel object (B). This process is repeated using new objects for Days 6 (2 hour delay), and on days 12 (5 minute delay) and 13 (2 hour delay). The testing for Days 12 and 13 evaluates mice after drug treatment is introduced (Figure 2). Exploration times are recorded and used to calculate a discrimination index $[\text{time spent with object A3} - \text{time spent with object B}] / [\text{total time exploring both objects}]$ for training and test sessions. Discrimination indices of 0 indicate equal exploration of both objects.

The paradigm depicted in Figure 1 enables testing of HAND and control mice using a within-subjects design, thereby allowing evaluation of memory prowess before and after treatment(s). Preliminary data showed that on Day 5, with a 5 minute delay between introducing the original objects and substitution of a new object, HAND mice do not discriminate the new object as well as controls. Similarly on Day 6, with a longer 2 hour delay between introducing the original objects and substitution of a new object, HAND mice did not significantly discriminate between the new object and the previously presented object, while the control mice did discriminate, and this deficit appears greater than on Day 5. Therefore, 5 and 6 days after IC injection of HIV-infected human MDM, HAND mice displayed an inability to recall an object presented 5 minutes and 2 hours earlier. These data suggest that, like humans who show cognitive dysfunction with HAND [10], mice show cognitive dysfunction with HAND, as reflected in our measurements of memory retention for objects. HAND mice and controls were then given a novel treatment for 6 days (Figure 2). Typically, treatments are given subcutaneously and can be provided in dosage schedules up to three times daily, depending on drug half-lives. We had previously shown that interferon (IFN)- α blockers, including a neutralizing antibody to IFN α and a biologic [B18R] that binds IFN α , when given simultaneously with IC injection of human MDM, mitigated behavioral manifestations and histopathological abnormalities in HAND mice [18, 25]. Preliminary data using the paradigm in Figure 1 suggest that B18R, when given after the first ORT, largely reverses behavioral abnormalities seen in untreated HAND mice during the second ORT given on days 12 and 13.

These preliminary studies suggest that the HAND mouse model can be used for understanding the brain and behavior consequences of HIV infection, as well as preclinical assessment of novel therapeutics. There are, however, limitations of the HAND mouse model. Of note, some of these limitations stem directly from limitations of knowledge regarding the human condition. Without correlative human autopsy studies to guide investigators in devising HAND models that portray accurate histopathological aspects of HIV infection of the brain, animal models or *in vitro* systems that solely depict histopathology, without behavioral correlation, are limited in translational value. Since human autopsy studies correlating histopathology with mild HAND are rare (see above), understanding the pathogenesis of HAND must primarily rely on clinical biomarkers of disease derived from imaging and tissue sources, such as cerebrospinal fluid and blood. The histopathology in HAND mice can be, and has been, correlated with abnormal behavior, which will hopefully drive human research to explore similar relationships, because to fully realize translational merit and impact of discoveries in preclinical models, we must understand the relationship between pathology and clinical expression of disease in humans.

Some have asserted that because human cells are injected into the brain parenchyma, the HAND mouse model includes traumatic features. However, the trauma is limited to a minimal area around the injection site, as can be seen in controls injected intracerebrally with saline. Our routine control mice, which are injected IC with uninfected human MDM, exhibit normal behavior when compared to mice that have been injected IC with saline. In addition, HAND mice have substantially more pathology around the injection site than control mice injected with uninfected human MDM, including large areas of gliosis that can even extend into the contralateral hemisphere. These histopathological and behavioral features in HAND mice can only be attributed to HIV-infection of MDM and their resultant effects on the mouse brain. Additional limitations of this HAND mouse model stem from the limited longevity of the xenografts and the lack of dissemination of HIV from the mouse periphery into the CNS. Therefore, long term effects of HIV infection of the brain and mechanisms of viral entry into the brain cannot be studied. Nevertheless, the model portrays mild cognitive deficits, which as we have argued, are perhaps the most important aspect of HAND to model, particularly when developing treatments that can reverse cognitive dysfunction.

In conclusion, the HAND mouse model has contributed a great deal to our understanding of the disease and targets for treatment. However, to truly push forward in science and pave the way for optimized preclinical models, we must acknowledge that there are no perfect animal models, and the HAND mouse model is no exception. Even experimental autoimmune encephalomyelitis, perhaps the most accepted rodent model of brain disease in the world, has significant drawbacks that are well described [26]. As exemplified in this article, it is clear that the HAND mouse model can serve as an excellent preclinical screening model for novel therapeutics, as well as for discovering driving mechanisms of the disease process and its progression. The general approach used in the model to characterize learning and memory, as well as to determine relationships amongst brain changes and cognition, can serve as a good template for investigators aiming to refine HAND models. NeuroAIDS investigators await with great anticipation the results of human HAND biomarker and autopsy studies that will guide us in the construction of more precise models. Indeed, the more we know about the human condition, the better we can model it to optimize discovery of mechanisms and avenues of novel treatments. Ultimately, the goal is to eradicate HIV, but until such treatments are found, adjunctive therapies that can be added to cART must be developed to minimize or eliminate this common and debilitating aspect of HIV brain infection. Its also quite possible that these adjunctive HAND therapies will be applicable to other cognitive disorders and neurodegenerative diseases.

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Object Recognition Testing

Figure 1.

Schematic of the Object Recognition Test. The triangles are representative of objects A1, A2 and A3, as stated in the text. The cube is representative of object B, as stated in the text.

Experimental Method

Figure 2.

Diagram of the timeline of ORT testing and treatment. After preparation of human MDM with or without HIV, mice are injected IC on Day 0. The first ORT includes days 5 and 6, immediately after treatment starts. The second ORT is days 12 and 13, and mice are sacrificed the following day.