REVIEW

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Animal models of HIV peripheral neuropathy

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ABSTRACT: The use of animal models in the study of HIV and AIDS has advanced our understanding of the underlying pathophysiologic mechanisms of infection. Of the multitude of HIV disease manifestations, peripheral neuropathy remains one of the most common long-term side effects. Several of the most important causes of peripheral neuropathy in AIDS patients include direct association with HIV infection with or without antiretroviral medication and infection with opportunistic agents. Because the pathogenesis of these diseases are difficult to study in human patients, animal models have allowed for significant advancement in the understanding of the role of viral infection and the immune system in disease genesis. This review focuses on rodent, rabbit, feline and rhesus models used to study HIV-associated peripheral neuropathies, focusing specifically on sensory neuropathy and antiretroviral-associated neuropathies.

Infection with HIV and its associated treatments can manifest as a variety of peripheral neuropathies depending on stage of infection and antiretroviral drugs [1–3]. Peripheral neuropathy is the most common neurologic complication of HIV infection with a prevalence as high as 69.4% in infected patients [4]. Clinically, the term peripheral neuropathy is broad and covers a wide range of underlying pathologies. Sensory predominant distal symmetric polyneuropathy (DSP) is the most common type of peripheral neuropathy in AIDS patients, with the development of DSP correlating directly with HIV infection status. Although DSP is associated with HIV infection, the mechanisms of neurotoxicity and axonal degeneration are not completely understood; however, it is likely to be an indirect effect because viral infection of sensory neurons is lacking [5,6]. A second form of sensory neuropathy that can develop in HIV-positive patients is antiretroviral toxic neuropathy (ATN), which is secondary to nucleoside analogue reverse transcriptase (NRTI) inhibitor treatment and subsequent mitochondrial toxicity. These neurotoxic drugs are no longer widely used in developed countries [7,8]; however, the rate of HIV sensory neuropathies remains unsatisfactorily high despite the initiation of safer medicines [9,10]. Morphologically, DSP is characterized by the loss of intraepidermal nerve fibers (IENFs), nerve fiber swelling, mononuclear inflammation, distal degeneration of long axons in a ‘dying back’ pattern and concurrent damage to the dorsal root ganglia (DRGs) [11–24]. DRGs contain the cell bodies of primary afferent neurons that form sensory endings in the periphery, including IENF and transmit sensory information back to the CNS. DRGs are the link between the peripheral nerves and the spinal cord. DRGs are also sites of neuronal damage and are associated with prominent mononuclear cell infiltrates [25], macrophage activation [18] and viral replication [26,27]. The dying back pattern culminates in degeneration of the gracile fasciculus in the

KEYWORDS

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spinal cord, which carries information on fine touch, vibrations and proprioception to the brain stem. Pallor of the gracile tract is characterized by a loss of both axons and myelin primarily in the upper thoracic and cervical segments of the spinal cord, and has been described in several neuropathy studies [15,23,28].

Mechanisms underlying the pathogenesis of peripheral neuropathy are poorly characterized and are hindered by lack of an appropriate animal model. Animal models are crucial to better define the pathogenic mechanisms underlying peripheral nervous system (PNS) disease, especially with respect to viral tropism and infection of PNS cells, the role of inflammation and the effects of neurotherapies. These models also form an important framework for the development of novel therapeutic strategies. This review will discuss relevant animal models of HIV-peripheral neuropathy and ATN, focusing primarily on rodent, rabbit, feline and nonhuman primate animal models [18,21,29–39]. There have been numerous *in vitro* and *ex vivo* studies of mechanisms of HIV peripheral neuropathy and antiretroviral toxicity, but they will not be addressed in this review.

Mouse models

Although numerous retroviruses exist in rodents and have proved valuable models for studying the effects of retroviral associated neuropathies, no known rodent lentiviruses exist and therefore alternate strategies to study lentiviral pathogenesis in rodents have been developed. A nonlentiviral murine retrovirus has recently been used to develop a model for HIV sensory neuropathy and has shown some pathologic similarities to the human counterpart [40,41]. However, the bulk of the work done in mouse models has been in transgenic (Tg) mouse models. Initial research was focused on the expression of gp120 in astrocytes of Tg mice and resulted in similar pathologic findings as humans with HIV encephalitis including astrocytosis, neuronal degeneration and loss, and microglial proliferation [42]. More recent mouse modeling has attempted to recapitulate the sensory neuropathy commonly seen with HIV patients, which was unexplored in the early mouse work. Keswani *et al.* utilized the gp120 Tg mouse model to study the pathogenesis of HIV-associated distal sensory neuropathy [31]. The gp120 expression was under control of a GFAP promoter, which allowed for preferential expression in

unmyelinated C-fiber axons and was distributed throughout the PNS of the mice. This group failed to note any alterations in PNS pathology after 2 months; however, they did report that in animals 12–15 months of age there was a decreased in IENF density in the hindlimbs [31]. This would be consistent with the late-onset distal sensory neuropathy seen in human patients; however, further research is needed to determine the suitability of this model.

Mouse models of ATN have also been explored [31,43]. The gp120 Tg mouse model has been utilized to study for NRTI toxicity and gp120 Tg mice that were given daily didanosine (ddI) developed distal degeneration of sensory nerve fibers with no alterations in motor nerve function [31]. Wild-type mice dosed with ddI in the Keswani *et al.* [31] study failed to develop any lesions indicative of ATN; however, more recent models of ATN in mice have been developed. C57BL/6J mice dosed with stavudine (d4T) develop tactile allodynia after 24 h of drug administration and had persistently decreased withdrawal thresholds [43]. Studies of d4T toxicity in these mice indicated alterations in the *Gan1* gene, which encodes the protein gigaxonin and is altered in some human motor and sensory neuropathies. Its role in the genesis of the peripheral neuropathy remains as yet undefined; however, it may be of potential significance.

Rat models

Similar to lesions seen in the rhesus macaque model (discussed below), HIV-gp120 is associated with macrophage infiltration in the DRG of experimental rats [32,33]. Similar infiltrates are noted in the peripheral nerve at the site of application [32,33]. The damage that is associated with gp120 includes microgliosis in the spinal cord; however, a similar increase in astrocytes has not been reported [32–33,44]. Microarray analysis of DRGs from experimental rats indicates marked increased expression of a variety of genes pro- and anti-inflammatory genes [45]. Administration of gp120 in the rat also leads to upregulation of MCP1/CCR2 signaling in neurons of affected DRG [34]. This may indicate another route that ganglion cells can be damaged during the pathogenesis of the disease (i.e., degenerating neurons release MCP-1, which damages adjacent, currently unaffected, neurons). Lastly, gp120 also upregulates TNF α production in spinal astrocytes and microglia, and is associated with the development of mechanical allodynia [46].

Early rat models of distal sensory neuropathy studied various drugs that were administered intradermally or intravenously [47–49]. These included the antiretroviral zalcitabine (ddC), ddI and d4T. Other drugs used included L-NMA, suramin and TMB-8. Administration of these drugs is associated with the development of mechanical hypersensitivity and allodynia [48,49]. This model also revealed a potential role of caspases and mitochondrial electron transport abnormalities in the development of the peripheral nerve lesions [47]. Intravenous injection of d4T in rats also leads to mechanical hypersensitivity in the paw, and decreased IENF density and increased immunoreactivity for calcitonin gene-related peptide in the spinal cord, suggesting damage to the associated dorsal root ganglion neurons [50]. No significant inflammation or gliosis was noted in the animals solely treated with d4T, further suggesting the important role that HIV presumably plays in worsening the disease pathology and progression [50].

More recent rat models have focused on the role that the potent antiretroviral, ddC, and HIV-gp120 play in the genesis of lesions [32,33]. Gel foam containing HIV-gp120 administered directly to the sciatic nerve in the rat caused increased transcription of ATF3; a stress related transcription factor, as well as various neuro-peptides in sensory neurons [32]. GFAP expression is similarly increased, further corroborating the role that satellite cells have in the development of sensory neuropathies. Interestingly, no overt changes were noted in protein expression within neurons of affected animals [32]. Both treatment with ddC and HIV-gp120 are also associated with increased expression of CCL2 in the DRG of experimental animals [33]. That ddC and HIV-gp120 exacerbate lesion development in the peripheral nerves and DRG lends further support the hypothesis that the development of this disease is worse in patients that are both infected with HIV and are receiving antiretroviral therapy (ART).

IENF density is decreased both in animals dosed solely with ddC and those also given HIV-gp120 [32,33]. Control rats treated with ddC develop hindpaw mechanical hypersensitivity; however, altered stimuli to heat or cold have not been observed [32–34]. This lack of responsiveness to temperature stresses is similar to that seen in human patients with sensory neuropathy [51]. Rats given HIV-gp120 and concurrently treated with ddC have exacerbated mechanical

hypersensitivity as well as altered locomotor activity [32,33]. The hypersensitivity to mechanical stimuli in the rat can be ameliorated with treatment by gabapentin, morphine, diazepam and cannabinoid derivatives [49]. Interestingly, it is not sensitive to amitriptyline [49].

Rabbit model of ATN

In 1992, the first clinical, electrophysiologic and pathologic description of a rabbit animal model of a peripheral neuropathy induced by a nucleoside analog was published [52]. New Zealand White rabbits were given ddC by oral intubation and showed hindlimb paresis and/or gait abnormalities after 16 weeks of treatment [52,53]. In a follow-up study, transmission electron microscopy revealed a prominent ultrastructural change induced by ddC in sciatic nerve and ventral root [53]. Additional studies showed the peripheral neuropathy caused by ddC in rabbits was characterized as a myelinopathy of the proximal portion of the nerve fibers and as an axonopathy involving both proximal and distal fibers [54]. ddC also had an effect on structure and function of Schwann cell mitochondria [55]. In 1995 a study showed that although peripheral neuropathy has been reported in rabbits with ddC, based on clinical observations, electrophysiological measurements, and light and electron microscopy, no evidence of peripheral neurotoxicity was observed in rabbits given either ddI or d4T [56]. Rabbits as animal models of HIV peripheral neuropathy have not been used since the 1990s.

FIV model of HIV neuropathy

FIV is a naturally occurring [57], immunosuppressive lentiviral infection of cats that recapitulates many of the pathologies of HIV including lesions within the PNS [18,21,30,39]. Like HIV, FIV infection has similar cellular targets, including CD4⁺ and CD8⁺ lymphocytes and monocytes [58,59], and both use CXCR4 as a coreceptor [60]. Differences specific to FIV include a more simplified virus structure, different primary receptor (FIV uses CD134 instead of CD4) [61], the ability to infect B lymphocytes [58] and reduced severity of neuropathogenesis.

The Power laboratory has established a model in which infection using a chimeric infectious molecular clone, FIV-Ch, containing the full-length V1CSF *env* on a Petaluma background, induces neurologic and immune compromise in neonatally animals within 12 weeks of

infection [18]. FIV infection results in pathological events in the PNS similar to HIV including alterations in skin, and DRG, and changes in epidermal nerve fiber densities. In contrast to the scattered numbers of CD3⁺ cells seen in the DRGs of humans, rhesus and pigtailed macaques (see below) [29,36–37], infected cats have high levels of CD3⁺ T cells in the DRGs suggesting that T cells play an active role in FIV-induced damage [62]. Within the DRGs and nerves, FIV infection is associated with increases in activated macrophages as well as elevated levels of TNF α RNA. The FIV model has been helpful in elucidating potential mechanisms of neurotoxicity of viral infection [21,62] and used to evaluate the neurotoxicity of ART [30] and has confirmed the earlier work demonstrating the mitochondrial dysfunction by antiretroviral drugs. In FIV-infected cats, ddI treatment induced ATN in combination with DRG neuronal injury. ddI treatment lowered FIV loads; however, DRG FIV load was not reduced.

SIV infection of nonhuman primates as models of HIV sensory neuropathy

Genetically similar to HIV, the SIV targets CD4⁺ lymphocytes, monocytes and macrophages [63–68]. Like HIV, SIV gp120 also binds to the coreceptor CCR5. Macaque immune responses to SIV infection closely resemble immune responses in HIV-infected individuals. Although macaque models have been widely used in AIDS pathogenesis and vaccine studies, they have only recently been used to model HIV peripheral neuropathy.

• Pigtail macaques

An SIV model developed at John Hopkins University utilizes pigtailed macaques (*Macaca nemestrina*) infected with a neurovirulent molecular clone SIV/17E-Fr and an immunosuppressive virus swarm SIV/Delta B670. This model results in rapid CNS disease, where most animals develop SIV encephalitis (SIVE) by 84 days postinfection [36–37,69–70]. Recently Mankowski and colleagues have utilized SIV-infected pigtail macaques to study the pathology of peripheral neuropathy. In their initial studies, they showed that SIV replication is predominantly maintained in macrophages resident to the ganglia, and that trigeminal ganglia also had abundant CD68⁺ macrophages, neuronal loss and neuronophagia [37]. More recently they have observed similar changes in lumbar

DRG [36]. Similar to HIV and FIV infection, SIV-infected pigtail macaques had decreased IENF densities compared with age-matched controls. In addition, studies showed decreased C-fiber conduction velocity, both of which correlated to macrophage activation and neuronal density in lumbar DRG [36,37]. Distal axonal degeneration (as evidenced by reduced IENFs) was associated with mitochondrial dysfunction and increased oxidative stress in distal, but not proximal nerves [36].

The Hopkins group has also used the SIV-infected pigtails to determine whether SIV infection can influence the capacity of peripheral nerves to regenerate [36–38,69–71]. In order to do so, they have developed an excisional intracutaneous axotomy model where 3-mm serial skin punches are performed that transect the epidermal axons and allow subsequent postaxotomy epidermal nerve regrowth into the healing region. After 70 days of SIV infection a 5-mm punch is used to remove the previous 3-mm incision sites. The sprouting and normal nerve fiber lengths can be measured and used to devise a ratio between the two lengths. SIV-infection delayed epidermal nerve fiber regeneration and remodeling of new sprouts [70].

A recent study using SIV-infected pigtailed macaques showed impaired mitochondrial function from sural nerves compared proximal nerve segments [38]. The impairment was most prominent in mitochondria isolated from animals that also received ART treatment, suggesting additive mitochondrial toxicity of antiretroviral drugs [38]. These findings were correlated with increased mutation rate in mitochondrial DNA in distal nerves in HIV-associated sensory neuropathy (HIV-SN) patients validating the usefulness of the animal model [38]. The role of neurotoxic ART on the development of ATN in the pigtail macaques or the role of ART in peripheral nerve fiber regeneration has not been fully explored.

A recent study presented at the 2014 Conference on Retroviruses and Opportunistic Infections demonstrated the use of corneal nerve assessment in an SIV model of peripheral neuropathy. A decrease in corneal nerve fiber density concomitant with increases SIV RNA and CD68-positive macrophages in trigeminal ganglia was seen in SIV-infected macaques. This study reiterates the importance of animal models and how they may be used to develop new methods of detection or to monitor HIV neuropathy in humans [71].

• Rhesus macaques

Rhesus macaques (*Macaca mulatta*) are the most utilized nonhuman primate model for AIDS. Rhesus macaques infected with SIVmac251 typically progress to AIDS 1–2 years after infection (normal progressors) with an incidence of SIVE of 18% (16/89) [72]. Studies conducted by our laboratory and collaborators at Harvard Medical School have utilized a CD8-specific mouse–human chimeric antibody (cM-T807) to deplete CD8⁺ lymphocytes in rhesus macaques. After CD8 depleting animals on days 6, 8 and 12 postinfection (dpi), greater than 90% of the treated animals are persistently (>28 dpi with CD8⁺ cells remaining depleted) CD8 lymphocyte depleted [73–75]. Of these, approximately 78% develop SIVE (mean time to death 90 days; n = 22) [76]. We have recently [29] used this well-defined SIV infection model [29,68,74–81] to study PNS disease. In this model of HIV DSP, we find that macrophage activation and recruitment are critical components of DRG pathology [29]. Importantly, we demonstrated that this rapidly progressing CD8 depletion model accurately mirrors the peripheral nerve pathology from non-CD8-depleted SIV-infected macaques, but results in more severe DRG pathogenesis [29]. Our observations of morphologic and immunohistochemical alterations in monocytes/macrophages within the DRGs of SIV-infected CD8 lymphocyte-depleted macaques recapitulates lesions of sensory neuropathy in a shorter time frame of AIDS progression (3–4 months in CD8 T-cell-depleted macaques compared with greater than 1 year in conventional nondepleted macaques, and over 10 years in humans). Moderate to severe DRG pathology was found in the SIV-infected CD8 depleted macaques, which was characterized by abundant infiltration of mononuclear cells and formation of Nageotte nodules substantiating the observation that neuronal loss occurs in the DRGs of SIV-infected macaques [29]. Our findings are consistent with other reports describing ganglionitis with neuronal loss, neuronophagia and presence of SIV-infected macrophages in the accelerated SIV-infected pigtail macaque model [36,37]. Importantly, we show that numbers of CD3⁺ and CD8⁺ T cells were not significantly elevated in ganglia of non-CD8-depleted animals [29]. This is consistent with reports in pigtail macaques (described above) and humans that show minimal T lymphocytes in DRGs [15,37,82].

The CD8 depletion rhesus SIV infection model is a compressed window of the human disease that recapitulates viral infection, immune response to virus, macrophage traffic, peripheral nerve damage, IENF loss and DRG inflammation. SIV-infected rhesus macaques develop changes closely resembling those seen in HIV-infected patients including decline in IENF densities, DRG satellitosis, presence of Nageotte nodules in DRGs, neuronophagia, increased numbers of CD68⁺ macrophages and abundant viral replication [29]. Although the etiology of HIV-SN is not well defined, based on the CD8 depletion macaque model, peripheral immune activation including activated monocyte and macrophages play an important role. Both HIV-infected patients and CD8 depleted monkeys have IENF loss and DRG pathology. Thus, the model is similar in all respects to that seen in humans, but faster and more reproducible. The timing of infection can be controlled, pathology can be predicted, and as a result PNS pathogenesis and drug efficacy can be better studied.

We have also shown SIV infection in CD8-depleted rhesus alters CGRP, which is a potent vasodilatory neuropeptide that contributes to a variety of normal sensory, vasoregulatory and epidermal functions, but is also implicated in a variety of chronic pain conditions where it is expressed at high levels in the skin. All SIV-infected animals tested showed some degree of increased CGRP among the hypothenar region epidermal keratinocytes. Importantly, not all animals had the same degree of CGRP, consistent with the fact that they had different rates of disease progression with varying degrees of severity [83]. These results show that the SIV model may be useful to examine pathogenesis of pain associated with HIV infection.

• Neuropathies associated with opportunistic infections

The late stage of AIDS is accompanied by infection with and/or recrudescence of a variety of opportunistic agents. Most are not associated with nervous system disease; however, CMV infection in humans AIDS patients is associated with widespread inflammation and damage to peripheral nerves [84]. Typical pathologic findings include neutrophil-rich inflammation with necrosis, and axonal and myelin loss with the clinical features of polyneuropathy. CMV-infected fibroblasts, macrophages and endoneurial cells are commonly noted [84]. A similar phenomenon has also

EXECUTIVE SUMMARY**Mouse models**

- Primary observations: no naturally occurring rodent lentiviruses have resulted in using transgenic mouse models that express gp120; both gp120 and wild-type mice have been used to study the effects of antiretroviral therapy (ART).
- Unresolved issues: correlating clinical evidence of peripheral neuropathy in the mouse with underlying histopathologic and immunopathologic changes has been difficult to produce, including the early development of peripheral nervous system pathology.
- Future work: to better define the immunopathologic correlates of peripheral neuropathy in the transgenic mouse models; to develop novel transgenic mouse models to test therapies.

Rat models

- Primary observations: similar to mice, there is no naturally occurring lentivirus in rats and therefore work has been concentrated on the injection of gp120 with or without concurrent ART; macrophage infiltration is a key component of the inflammatory response to the injection of gp120.
- Unresolved issues: better characterization of the genes that are up- or down-regulated in the experimental rats; further description of the macrophages and their subtypes are also needed.
- Future work: expanding work in understanding the inflammatory cell infiltrate that accompanies injection of gp120 and the role that it plays in disease progression and exacerbation.

Rabbit model of ATN

- Primary observations: zalcitabine- but not didanosine- or stavudine-induced peripheral neuropathy has been reported in New Zealand White rabbits; rabbits developed hindlimb paresis and/or gait abnormalities as well as ultrastructural changes.
- Future work: the rabbit model is not currently used in the study of HIV neuropathy.

FIV model of HIV neuropathy

- Primary observations: FIV infection results in pathological events in the PNS similar to HIV including alterations in skin and dorsal root ganglia (DRGs), and changes in epidermal nerve fiber densities; the FIV model has been helpful in elucidating potential mechanisms of neurotoxicity of viral infection and evaluating the neurotoxicity of ART drugs.
- Unresolved issues: FIV utilizes CD134 coreceptor instead of CD4, infects CD8⁺ T cells and B lymphocytes, and has reduced severity of neuropathogenesis.
- Future work: understanding the role that pro- and anti-inflammatory gene expression plays in the immunopathology, leukocyte trafficking and progression of neuropathy in FIV-infected cats.

SIV infection of nonhuman primates as models of HIV sensory neuropathy

- Primary observations: SIV infection of both pigtailed and rhesus macaques results in DRG pathology, including increased macrophage infiltration, viral infection, neuronophagia and decrease in intraepidermal nerve fiber density, similar to humans.
- Unresolved issues: study of antiretroviral toxic neuropathy in macaques has not been carried out.
- Future work: the SIV model will continue to be important in dissecting the pathogenesis of HIV-associated sensory neuropathy by examining the role of infiltrating and resident monocyte/macrophage in DRG pathology and neuronal loss, viral infection, and their link to intraepidermal nerve fiber density loss.

Conclusion

- There are currently no therapy for prevention or treatment of HIV-associated sensory neuropathy.
- We need to focus on development of animal models that mimic the human condition more reliably and the use of outcome measures that are also used in the clinical setting (i.e., intraepidermal nerve fiber densities).
- Animal models are essential for defining mechanisms of neuronal injury, studying synergistic effects of HIV and neurotoxic ART, and developing of new therapeutic approaches and adjunctive therapies.

been observed in SIV-infected rhesus macaques with recrudescence rhesus CMV infection causing lesions in peripheral nerves and nerve roots along the spinal cord [85]. Some lesions can affect the DRG; however, functional tests to determine if clinical features of sensory neuropathy develop have not been explored [MILLER AD, UNPUBLISHED DATA]. Rhesus CMV likely represents an underutilized model for opportunistic infection-associated peripheral neuropathy and further development of this model is warranted in SIV-infected rhesus macaque with CMV recrudescence.

Other potential animal models

There are relatively few other lentiviruses known to infect mammals. Outside of HIV SIV, and FIV, the other significant mammalian lentiviruses are Maedi/Visna virus (MVV) in sheep, caprine arthritis and encephalitis virus (CAEV) in goats, equine infectious anemia in horses, and bovine immunodeficiency virus in cattle and experimentally in rabbits. Of these, only MVV and CAEV cause extensive CNS disease, which consists of lymphocytic inflammation with virus dissemination and propagation occurring within macrophages [86]. PNS lesions are not described in either MVV or CAEV and further work needs to be carried out to determine if either of these viruses might be suitable models for lentiviral-associated peripheral neuropathy.

Conclusion & future perspective

Small animal models, such as mice and rats, are advantageous as they have high reproduction rate, low maintenance cost and provide an opportunity for rapid screening of potential therapies. However, they are more genetically distant from humans and the lack of a rodent lentivirus hinders the exploration of naturally occurring disease.

Potentially more relevant animal models of HIV sensory neuropathy have gained greater traction over the last decade as these models

mimic the human condition more reliably and can be studied using outcome measures that are used in human clinical medicine. IENF densities are currently used in the clinic and can be used in clinical trials as an outcome measure. Therefore, IENF measurement is an important tool to use in animal models. Cats and large animal models, such as nonhuman primates, are beneficial since they allow for these kinds of serial measurements as well as the ability to evaluate potential pathologic mechanisms. SIV infection of nonhuman primates closely resembles HIV infection in humans with respect susceptible cell types, coreceptors and progression to AIDS, partially due to their close phylogenetic relationship. The SIV model will continue to be important in dissecting the pathogenesis of HIV-SN by examining the role of infiltrating and resident monocyte/macrophage in DRG pathology and neuronal loss, viral infection and their link to IENF density loss.

Further study based in animal models of HIV peripheral neuropathy will be critical for our understanding of the consequences of infection. Animal models will be essential for defining mechanisms or neuronal injury, studying synergistic effects of HIV and neurotoxic ART, and developing of new therapeutic approaches and adjunctive therapies. Knowledge of the pathogenesis of HIV sensory neuropathy will continue to improve as existing models are refined and new ones are developed.

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