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Battle of Animal Models

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Abstract

This is a brief summary of the animal models session held during the 12th Annual Meeting of the Society on NeuroImmune Pharmacology, Santa Fe, NM. This session provided important information for participants on availability and utility of animal models for the studies of HIV-1 central nervous system infection and drug abuse. It highlighted animal model relevance to human disease/condition, their utility for the studies of pathogenesis, potential importance for the development of therapeutics, and demonstrated limitations/pitfalls.

A special session on development and utility of different animal models for drug abuse studies and neuroAIDS was held during the 12th SNIP meeting in Santa Fe, NM (April 5–9, 2006). The following is a brief summary of the presentations of Drs. Claire Gavériaux-Ruff (Strasbourg, France), David Volsky (New York, USA), Sulie Chang (New Jersey, USA), Larisa Poluektova and Yuri Persidsky (Nebraska, USA), Tricia Burdo (California, USA), Anil Kumar (Puerto Rico), and Steve Hendriksen (California, USA).

Opiate Receptor Knockout Mouse Model

Dr. Gavériaux-Ruff

addressed utility of mice lacking opioid receptors and peptides in studies of immune and behavioral abnormalities in drug addiction. The opioid system is composed of three receptor types, mu, delta, and kappa, activated by three families of endogenous opioid peptides, endorphins, enkephalins, and dynorphins. The opioid system controls pain perception, stress responses, addictive behaviors, and several functions including digestion, endocrine responses, and immunity. This system is the target of exogenous opiates, with morphine as a classic example, inducing analgesia and addiction.

Mice lacking opioid receptors and peptides (knockouts, KO) were generated by gene targeting. Mouse phenotyping in the absence of drugs showed that mu- and delta-opioid receptor mutant mice display opposite phenotypes in behavioral models for emotional responses such as anxiety, and highlighted the role of the delta-receptor to lower anxiety. All three opioid receptors modulate pain perception and stress responses (Contet et al., 2006). Similarly, opioid peptide KOs show altered anxiety, tetrahydrocannabinol (THC) dependence (enkephalin-KO), alcohol addiction (beta-endorphin-KO), and THC dysphoria (dynorphin-KO). In mu-receptor KO animals, all morphine effects including analgesia, reward, dependence, and side effects are abolished. Interestingly, the decreased addictive properties of THC, alcohol, and nicotine

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demonstrated that this receptor is a central element in addiction [for review, see (Kieffer and Gaveriaux-Ruff, 2002)].

The mutant mice allowed exploration of a role for the different components of the opioid system in immunity. First, studies in absence of drugs indicated changes in lymphocyte numbers and augmented antibody response mainly in kappa-receptor KOs (Gaveriaux-Ruff et al., 2003), and augmented lymphocyte proliferation and levels of cytokines in beta-endorphin KOs (Refojo et al., 2002). Mu-receptor mutants displayed enhanced inflammation in a model for inflammatory bowel disease (Philippe et al., 2003); whereas, enkephalin-KOs showed diminished inflammatory responses in the lung (Hook et al., 2000). Morphine-immune effects were abolished in the mu-receptor mutants (Gaveriaux-Ruff et al., 1998).

Together, genetic animal models demonstrate utility for studies of pathogenesis in emotional, addictive, and inflammatory diseases. Opioid pharmacology in wild type and KO animals has identified opioid targets at a molecular level *in vivo*. The evidence of new phenotypes in these mice will lead to the development of new therapeutics, as exemplified for delta-receptors and anxiety, or mu-receptors and inflammation. A limitation for interpretation of data from the classical KO is that gene inactivation occurs at the embryonic stem cell stage and in the whole body. This is now overcome with the development of conditional KOs where genes can be inactivated in an organ/tissue in a time-controlled manner.

EcoHIV Mouse Model

Dr. Volsky

presented data on the EcoHIV mouse model (Nitkiewicz et al., 2004; Potash et al., 2005). Dr. Volsky's group replaced the coding region of gp120 in HIV-1/NL4-3 or HIV-1/NDK with that of gp80 from ecotropic murine leukemia virus (Eco), creating chimeric HIV-1 EcoHIV and EcoNDK with exclusive tropism to rodent cells. Adult, immunocompetent mice were readily susceptible to infection by a single intravenous or intraperitoneal inoculation of EcoHIV or EcoNDK. Virus burden *ex vivo* was assessed quantitatively by quantitative polymerase chain reaction assays for genomic DNA, genomic and spliced RNA, and p24 antigen by ELISA. At the peak of infection, approximately 1%–3% of spleen cells and up to 10% of peritoneal macrophages carried HIV DNA and significant viral RNA; however, virus burden in the brain was lower. Infection was productive as shown by virus passage from spleen cells in culture, by detection of p24 in lymphocytes and macrophages *ex vivo*, and by induction of antibodies to HIV-1 *gag* and *tat*. EcoNDK entered the brain within 3 weeks of infection of adult mice and increased expression of genes induced by infection (MCP-1, STAT1, IL-1 β , and complement component C3) was found in brain tissue. Six months after infection, viral DNA and transcripts were still present in spleen cells and macrophages, albeit at lower levels than early after infection, indicating effective antiviral immune responses. This model of systemic infection offers a unique opportunity to investigate drugs targeting HIV-1 with virus detection within days of infection and maintenance of active virus replication for months.

EcoHIV infection of mice reproduces important elements of HIV-1 infection of humans including persistent systemic infection, tropism to T cells and macrophages, antiviral immune responses, neuroinvasiveness, and induction of expression of genes associated with HIV-1 infection in humans. However, EcoHIV enters cells through a different receptor than does HIV-1, the mouse may not fully reproduce human responses, and EcoHIV lacks gp120. To address the latter concerns, Dr. Volsky's laboratory currently employs mice fully transgenic for human histocompatibility antigens, and pathogenesis-related domains of gp120 are inserted into the EcoHIV genome. Despite these limitations, EcoHIV is the first chimeric HIV-1 capable of "natural" primary infection of mice permitting realistic modeling of HIV-1 infection of

humans for convenient and safe investigation of HIV-1 pathogenesis, therapy, and potential vaccines.

Rat Transgenic Model

Dr. Chang

described potential utility of a rat transgenic model (Tg) in studies of opiates and HIV-1 infection. The HIV-1 Tg rat carries a *gag-pol*-deleted HIV-1 genome under the control of the HIV-1 viral promoter and expresses 7 of the 9 HIV genes (Reid W, 2001). While there is no viral replication in the HIV-1 Tg rat model, viral proteins are expressed in various organs (Reid W, 2001) and blood (Reid W, 2001; Mazzucchelli et al., 2004). The HIV-1 Tg rats develop pathologic alterations reminiscent of humans infected with HIV-1 [expression of viral genes, abnormal immune responses, pathologies with advancing age (Reid W, 2001; Mazzucchelli et al., 2004), and renal failure (Ray PE, 2003)]. Progressive neurobehavioral and neuropathologic changes were seen within the brain parenchyma of these animals (Reid W, 2001). The development of various manifestations of human HIV infection in the HIV Tg rat, without viral replication, indicates that the presence of viral proteins in the host is sufficient to affect the targets cells. The HIV Tg rat appears to mimic the condition of patients on anti-retroviral therapy with controlled viral replication, but persistent HIV infection.

While numerous studies have shown that opioid use and abuse are involved in the immunopathogenesis of HIV-1 infection and AIDS progression (Donahoe and Vlahov, 1998; Nath A, 2002; Donahoe, 2004; Kumar et al., 2004; Hauser KF, 2005), little is known about the interactive effects of opioids and bacterial endotoxins in the course of HIV infection. Dr. Chang demonstrated that the expression of the mu opioid receptor (MOR) was significantly higher in HIV Tg rats than that in control animals due to circulating viral protein, gp120. Systemic treatment with lipopolysaccharide (LPS, 1.25 mg/kg, 24 h) mimicking the effect of bacterial infections resulted in up-regulation of the expression of MOR in both HIV-1 Tg and control rats; however, the extent of up-regulation was significantly higher in the HIV-1 Tg rats. Dr. Chang showed that systemic injection of a non-pyrogenic dosage of LPS resulted in 38- and 7-fold increase of serum TNF- α and IL-1 β levels, respectively, as compared to control group. These changes did not correlate with LPS-induced leukocyte-endothelial adhesion, possibly reflecting the dysfunction of immune responses observed in patients infected with HIV. The HIV-1 Tg rat carrying a *gag-pol*-deleted HIV-1 genome could be an ideal small animal model to investigate the interaction of opioid and bacterial endotoxin in the course of persistent HIV infection.

Mouse-Human Chimeric Models

Drs. Poluektova and Persidsky

presented data on mouse-human chimeric models using severe combined immune deficiency (SCID) mice for studies of neuropathogenesis. The neurologic complications of HIV-1 infection are related to the productive viral replication in brain resident microglia and perivascular macrophages. The most reliable approach to study neuropathogenesis of HIV-1 infection is injection of human peripheral blood mononuclear cells or macrophages into the brain of 4–6 weeks old immune deficient mice (Persidsky et al., 1996; Persidsky et al., 1997). Long-time survival of macrophages and productive infection with the release of human and mouse inflammatory substances resulted in neurotoxic activities, behavior abnormalities, neuropathologic changes reminiscent of HIV-1 encephalitis in humans, and neuronal damage (Zink et al., 2002). Over the last ten years, this model was used extensively to identify neurotoxic factors, address the role of different viral strains, and test a number of anti-retroviral and adjunct therapeutics (Limoges et al., 2000; Limoges et al., 2001; Persidsky et al., 2001; Dou et al., 2003; Nukuna et al., 2004; Dou et al., 2005).

It is well accepted that macrophages that are infected and activated are the major source of toxicity. However, the initial SCID model is missing the components of adaptive immunity that have a dual role in HIV-1 neuropathogenesis. A strong association between HIV and profound immunodeficiency led to the conclusion that a lack of effective T cell immune responses is associated with ongoing viral production in the brain. Neuropathological analyses revealed increased numbers of CD8⁺ lymphocytes within the neuropil next to virus-infected macrophages in brains of individuals with HIV as compared to brain tissue from seropositive patients without evidence of encephalitis (Potula et al., 2005). In this setting, the inflammatory cytokines and cytotoxic molecules (like granzyme B or perforin) released by these cells upon activation could contribute to the neurological sequelae of infection. The HIV-1-specific cytotoxic lymphocyte (CTL) responses were studied in a non-obese diabetic (NOD)-SCID mouse model of HIV. HIV-1-infected macrophages were injected into the basal ganglia after syngeneic immune reconstitution by human peripheral blood lymphocytes (PBL) to generate human PBL-NOD/SCID HIV mice (Poluektova et al., 2002; Poluektova et al., 2004). Engrafted T lymphocytes produced HIV-1*gag*- and HIV-1*pol*-specific CTLs against virus-infected brain monocyte-derived macrophages (MDM) within 7 days. This was demonstrated by tetramer staining of human PBL in mouse spleens and by IFN- γ ELISPOT. CD8, granzyme B, HLA-DR, and CD45R0 T cells migrated to and were in contact with human MDM in brain areas where infected macrophages were abundant. The numbers of productively infected MDM were markedly reduced (>85%) during 2 weeks of observation. However, the generated CTL were not able to completely eliminate HIV-1-infected cells, and animals developed viremia (Poluektova et al., 2004). This model allowed investigation of antiviral and immune modulating drugs improving antiviral responses in the brain (Potula et al., 2005) and effects of co-factors (like alcohol abuse) of HIV-1 central nervous system (CNS) infection (Potula et al., 2006). The limitations of this model are graft-versus host reaction mediated by mature PBL and over activation of human cells in a mouse environment. All of those were weakened by selection of the low reactive donors for transplantation. The models serve as unique tools to study the role of effector lymphocytes and their expansion in mouse brain environment, cell migration across the blood-brain barrier, cell-cell interaction and consequences of T cell responses in the brain environment.

The “dream” mouse-based model will be an animal carrying a functional human peripheral immune system with brain repopulation by human microglial cells. The first step in this direction was achieved by reconstitution of Balb/c-Rag2^{-/-}gc^{-/-} or NOD/SCIDIL-2Rgc^{null} mice with human hematopoietic stem cells (Traggiai et al., 2004; Persidsky et al., 2005). Preliminary data using this approach were presented at the conference (Gorantla et al., 2006).

SIV Models

Dr. Burdo

presented her work exploring the effect of simian immunodeficiency virus (SIV) infection on monkeys of different origins (Burdo et al., 2005). Most studies of SIV pathogenesis to date have used rhesus macaques of Indian origin, which have proven quite valuable as an experimental model of HIV infection of people. Serial passage of virus is a method used in SIV studies in order to select desired characteristics in viral stocks in monkeys (Sharma et al., 1992; Watry et al., 1995; Joag et al., 1996; Reimann et al., 1996; Holterman et al., 1999). For well-studied pathogenic viruses, such as the SIVmac251 stock or the SIVmac239 molecular clone, development of simian AIDS is observed in an experimentally useful time frame. Two distinct patterns of disease course, known as rapid-progression and normal, are found in this model. For example, in a survival analysis of animals infected with pathogenic SIV, it was observed that approximately one-third of each group survived 200 days or less; the remaining two-thirds developed disease at a later time point, with half being sacrificed due to terminal disease occurring approximately two years following infection (Johnson et al., 2003).

This model of SIV infection of Indian origin rhesus macaques has been useful for studying disease pathogenesis, treatment, and vaccines. However, an impediment to research is a severe shortage of India-derived rhesus monkeys (Cohen, 2000; 2003). Chinese rhesus monkeys are available, but the suitability of these animals for AIDS research has been questioned. Chinese monkeys appear to survive longer following SIV infection than do Indian monkeys (Ling et al., 2002; Trichel et al., 2002), but the disease course has not been well characterized in SIV-infected Chinese rhesus monkeys. In SIV-infected Indian rhesus monkeys, survival is inversely correlated with steady-state plasma viral load (Watson et al., 1997; Ten Haaf et al., 1998; Smith et al., 1999). However in one experiment, infection of Chinese rhesus monkeys revealed a lower plasma viral load than did infection of Indian rhesus monkeys (Ling et al., 2002). In another experiment the two groups of animals were indistinguishable (Marthas et al., 2001).

Indian vs Chinese Monkey Models

Dr. Fox's laboratory has used a stock derived from serial passage of microglia in Indian monkeys, SIVmac182, in their studies of neuropathogenesis in rhesus macaques (Lane et al., 1995; Watry et al., 1995; Fox et al., 2000; Marcondes et al., 2003; Roberts et al., 2003). Using this stock, the steady-state plasma viral loads achieved in Indian rhesus monkeys were significantly higher than those found in Chinese monkeys. However since this stock, as well as most pathogenic SIVmac viruses, had been derived by serial passage in Indian monkeys, serial passages in Chinese monkeys were performed. Indeed, infection of a group of Chinese rhesus monkeys with this Chinese passaged stock resulted in a high steady-state plasma viral load, indistinguishable from the mean viral set points found by the laboratories described above, and by others (Parker et al., 2001) in Indian rhesus monkeys infected with Indian passaged SIV. Furthermore, by four months post-inoculation, two of nine animals developed simian AIDS, with both having SIV encephalitis.

Thus, an SIV stock that induced a high steady-state viremia, as well as simian AIDS, in Chinese macaques was developed. Even though there was a significant difference in the steady-state viral load of Chinese animals infected with Indian-derived virus versus those infected with the Chinese-passaged virus, only limited distinct changes in the sequence of the passaged virus were found (Burdo and Fox, unpublished data). The maintenance of these changes in viruses isolated separately from different monkeys indicate that they may be critical in the natural adaptation of the virus from Indian to Chinese monkeys, but the role of any specific change is not yet known.

There are several advantages to this model of AIDS utilizing Chinese rhesus macaques. First, SIV infection of Chinese macaques can be an efficacious animal model for the study of AIDS. Second, similar to HIV in humans, SIV pathogenesis in nonhuman primates is not limited by geographic origin, making Chinese animals a useful alternative to Indian rhesus macaques, which are in short supply (Cohen, 2000). Third, the availability of this Chinese-passaged viral stock, pathogenic in Chinese rhesus monkeys, can now be utilized in a variety of studies on SIV and AIDS pathogenesis.

This model can be used to study the interaction of drugs of abuse and SIV, as a paradigm for HIV infection in drug abusers, populations disproportionately affected by HIV. While the behavior associated with drug use can lead to greater exposure to HIV and thus infection, it is still not clear whether the effects of drugs on the body can increase one's susceptibility to infection, and/or affect the progression of disease. In the Fox laboratory, rhesus monkey models of methamphetamine (Madden et al., 2005) and ethanol (Katner et al., 2004) administration were developed. The study of SIV infection, in the presence of modulating factors such as drugs of abuse, will add not only to our knowledge on HIV pathogenesis, but also provide us with the tools to better treat those infected with HIV.

Nonhuman Primate Model

Dr. Kumar

presented his laboratory's nonhuman primate model for opioid effects on AIDS (Kumar et al., 2004; Kumar et al., 2006). Previously, two other groups reported divergent results of morphine, as a model opioid, on disease progression in SIV infected monkeys. In one, morphine appeared to exacerbate disease (Suzuki et al., 2002); whereas, in the other protection was found (Donahoe et al., 1993). In the study presented, half of the rhesus monkeys were first made dependent on morphine. For SIV infection, a mixed virus inoculum was used, combining SHIV(KU-1B), SHIV(89.6P), and SIV(17E-FR) in order to deplete circulating CD4⁺ T cells early after infection, allowing the development of simian AIDS in an experimentally useful time period, and providing a neurovirulent virus to be able to assess the effects of opioids on CNS infection.

Following infection, the peripheral CD4⁺ T cells were depleted more rapidly in the morphine-treated group than in the control group. The plasma viral load, although initially similar, was significantly higher two months after infection in the morphine treated group than in the control group. Interestingly, in the cerebrospinal fluid (CSF), the viral load was also significantly higher in the morphine treated group than in the control group beginning at week 6 post-inoculation. This study (Kumar et al., 2004), using a pathogenic virus and good-sized experimental and control groups provided evidence for a untoward effect of opioids on AIDS.

Further study revealed several intriguing findings (Kumar et al., 2006). Peripheral monocyte counts became elevated in the SIV-infected, morphine-treated animals concomitant with the development of disease. This elevation and development of simian AIDS occurred in four of the six morphine-treated animals and was accompanied by elevation of CCL2/MCP-1 in the plasma. The other two, as well as the SIV-infected non-morphine treated animals, did not develop AIDS in the first year following infection. Three of the four morphine-treated animals that developed AIDS had dramatic elevations of virus in the CSF, whereas the other three had low levels similar to the controls. Examination of viral genotype in the CSF revealed that SHIV (KU-1B) and SIV(17E-FR), but not SHIV(89.6P), were neuroinvasive.

These investigators have thus developed a system in which not only the effects of opiates on HIV can be studied in a reproducible primate model, but which also allows the study of the CNS, with certain viral genotypes capable of crossing into the CNS and, in many of the cases, replicating to quite high levels. This model can be used for further study of opiates effects on AIDS, for a more in depth investigation of the CNS for neuroAIDS, as well as for assessment of the effects of other drugs of abuse in this primate system.

FIV Model

Dr. Henriksen

presented a comparison of findings in SIV-infected monkeys, FIV-infected cats, and rodent models, with the overall themes that certain models are ideal for specific experiments, and that the combined knowledge one can draw through comparing and contrasting the models is great. For example, electrophysiological analysis of the CNS has proved to be a sensitive measure of CNS dysfunction in both the FIV/cat and SIV/monkey models (Phillips et al., 1994; Phillips et al., 1996; Prospero-Garcia et al., 1996; Horn et al., 1998).

A similar approach was then applied to the study of the interaction of drugs of abuse and HIV. Although the SIV/monkey model may be the closest to the human system, one is often hampered by relatively small sample sizes and limited number of conditions that can be tested. In the FIV/cat model, these limitations are not as profound, and different paradigms of drug administration have been tested, such as for stimulants and opiates, which have already been

studied in the FIV model (Barr et al., 2000; Phillips et al., 2000; Barr et al., 2003; Cloak et al., 2004).

In summary, several animal models are currently available for the study of HIV-1 neuropathogenesis, mechanisms of neuroinflammation and neurodegeneration associated with virus CNS infection, role of co-factors (like drug and alcohol abuse), and development of novel neuroprotective and anti-viral treatment paradigms. Selection of a specific model should take into account its limitations and ability to mimic certain aspects of neuroAIDS.

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