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Mini-Review

Animal models of human immunodeficiency virus infection

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Summary

The search for a model of HIV infection continues. While much of the initial work focussed on animal models of AIDS, more recent efforts have sought animal models of HIV infection in which one or more signs of AIDS may be reproduced. Most initial small animal modelling efforts were negative and many such efforts remain unpublished. In 1988, the Public Health Service (PHS) AIDS Animal Model Committee conducted a survey among PHS agencies to identify published and unpublished data on animal models of HIV. To date, the chimpanzee is the only animal to be reliably infected with HIV albeit without development of signs and symptoms normally associated with human AIDS. One recent study has shown the gibbon to be similarly susceptible to infection with HIV. Mice carrying a chimera of elements of the human immune system have been shown to support the growth of HIV and F_1 progeny of transgenic mice containing intact copies of HIV proviral DNA, have developed a disease that resembles some aspects of human AIDS. Rabbits, baboons and rhesus monkeys have also been shown to be infected under certain conditions and/or with selected strains of HIV but again without the development of AIDS symptomatology. This report briefly summarizes published and available unpublished data on these efforts to develop an animal model of HIV infection.

HIV-infection; Animal model; Rodent; Rabbit; Primate

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As a scientific, medical and ethical issue, the development of immunotherapeutic, chemotherapeutic and vaccination modalities is critical in the treatment and prevention of acquired immunodeficiency syndrome (AIDS) in man. From the initial identification of AIDS as a disease entity, progress has been hampered by the lack of a suitable animal model of the disease with which to evaluate and identify promising compounds or regimens. Before the establishment of a definitive viral etiology for AIDS, an intensive search was underway for animal models that would mimic the disease as seen in man. These models consisted primarily of infections of animal retroviruses, i.e., feline leukemia virus, type D retrovirus, maedi-visna virus and others (Desrosiers and Letvin, 1987). With the isolation and identification of a retrovirus (Barré-Sinoussi et al., 1983; Popovic et al., 1984), the human immunodeficiency virus (HIV), as the etiologic agent of AIDS the search now centers on animal models of HIV infection that mimic one or more signs of AIDS in humans.

HIV is a retrovirus of the lentivirus subfamily. It infects B cells and macrophages though it preferentially replicates in human T cells (Klatzmann et al., 1984), and HIV particles and antigens have been demonstrated in multinucleated giant cells, macrophages and, less frequently, in endothelial cells in the brain (Lantos et al., 1989); HIV presence in neurons is controversial (Fauci, 1988). Two related groups of HIV have been identified to date: HIV-1 associated with the AIDS pandemic and HIV-2, found primarily in portions of west Africa (Clavel et al., 1986). A number of non-human primate retroviruses, as exemplified by Simian immunodeficiency virus macaque (SIV_{mac}) (Daniel et al., 1985), as well as ungulate lentiviruses (Gonda et al., 1985, 1987; Sonigo et al., 1985; Chiu et al., 1985; Stephens et al., 1986) are related to HIV by amino acid and nucleic acid homologies in conserved regions of the gag and env proteins as well as by certain biological properties, i.e., macrophage tropisms that cause neurologic disease.

Infection with HIV, defined as the replication of detectable virus in the host and the development of antibody to HIV, either of which can be detected in blood, does not occur in some animals. Despite efforts to infect a wide variety of animals including nonhuman primates, rodents, felines and others, no totally satisfactory animal model of HIV infection (with development of one or more disease signs) has been described to date. HIV-1 infects chimpanzees and produces a persistent viremia and antibody development, but without apparent signs of immunodeficiency disease (Alter et al., 1984; Fultz et al., 1986). This report will summarize published data and some unpublished data (PHS AIDS Animal Model Survey, 1988) on the development of animal models of HIV infection.

Many questions related to prophylactic antivirals and vaccines or other immunoprophylactic modalities can best be answered in a reliable, suitable animal model that not only is capable of HIV infection, viral growth and specific immune response, but will also develop clinical signs of disease. Such a model will greatly accelerate the pace of HIV antiviral and vaccine development. Extensive resources have been and continue to be expended in pursuing the goal of a reliable, suitable animal model of HIV infection; therefore, the Public Health Service Animal Models Committee believed that negative data on animal model development should be published.

Rodents

Initial studies in which rodents were inoculated with HIV-containing or probable HIV-containing material were, at best, unpredictable. The results of a series of experiments begun in 1982, to identify a useful small animal model for HIV infection, are summarized below (unpublished data; Morrow et al., 1987). In early experiments peripheral mononuclear cells obtained from patients with AIDS or AIDS-related conditions were injected subcutaneously (SC) or intraperitoneally (IP) (PMC) (10^6 cells/0.1 ml fluid/animal) into newborn mice, rats, hamsters and guinea pigs. Other materials injected were whole blood, platelets, plasma, ultracentrifuged platelet pellets and spleen cell suspensions obtained from patients with Kaposi's sarcoma, lymphadenopathy or idiopathic thrombocytopenia purpura. Later, with the isolation and characterization of HIV-1, cell-free virus itself was used in a similar manner. Of the 30 rats, 16 hamsters and 14 guinea pigs inoculated, none demonstrated any unexplained pathology or mortality. Furthermore, attempts to culture blood and spleen cells were unsuccessful even after the spleen cells were stimulated with phytohemagglutinin for three days before culture.

More than 300 mice selected from approximately 50 litters of different strains were also inoculated. Mice injected with the clinical specimens had a significantly higher mortality (18.7 percent) than did the mice receiving like material from healthy subjects ($P<0.023>0.01$ chi-square test) (Morrow et al., 1987). Pathologic changes noted included runting, opportunistic infections (with pneumonitis, diarrhea and dermatologic conditions), tumors, and in some cases fibrotic spleens. A number of leukocyte abormalities also were identified, including abnormal neutrophil/lymphocyte ratio, eosinophilia, monocytosis, basophilia and others. No direct cause of these pathologic changes could be determined and neither HIV nor antibodies to it could be detected in the blood or serum of any mice. In addition, HIV was inoculated IP into six litters of newborn BALB/c and two litters of MRL/lpr mice (Morrow et al., 1987). A higher mortality, albeit not significant, was observed in experimental BALB/c mice than in control mice. Infectious HIV and seroconversion were not observed. An attempt to adapt a variant of HIV to BALB/c mice by serial passage at two week intervals, using two litters/passage, was also unsuccessful. The virus was never recovered from any of the new passages of mice, nor was seroconversion detected in any mice.

Wells et al. (1985) also carried out early experiments on mice, rats, guinea pigs and hamsters. Murine spleen cells stimulated with concanavalin A were cocultivated with HIV-1-infected human T cells or with the supernatant from infected T cell cultures. The spleen cells were maintained in culture with interleukin-2 (IL-2) and developed into a virus-producing cell line. BALB/c mice then were inoculated intravenously (IV) and IP with the spleen cells, the HIV produced by the spleen cells or the virus produced by human T-cell lines. Lewis rats were also inoculated IV. Inoculated mice failed to gain weight for periods as long as three months and showed some cellular immune function abnormalities, such as splenic lymphoid infiltration that obscured the normal cellular architecture. Antibodies also were detected by enzyme-linked immunosorbent assay (ELISA) and spleen cells were po-

sitive by immunofluorescence for HIV antigens. The rats had transient antibody responses.

More recently, mouse-human chimeras, in which elements of the human immune system have been successfully established in the mouse have shown the ability to support HIV growth in the chimeric tissue (Namikawa et al., 1988; Kamel-Reid and Dick, 1988), thus affording a small animal model (mouse) for HIV infection without, to date, development of disease symptomatology. One such model, SCID-hu mice engrafted with human fetal thymic or lymph node implants, were inoculated with HIV-1 (Namikawa et al., 1988). Graded doses of HIV were inoculated directly into the thymus or lymph node implants. Viral replication spread within the human transplants. Viral RNA transcripts were found in most infected cells but some cells had both viral transcripts and viral protein. Transgenic mice containing intact copies of the HIV proviral DNA have been constructed (Leonard et al., 1988). F_1 progeny of transgenic mice mated with nontransgenic mice have developed a disease that in some respects resembles features in human AIDS; infectious virus has been recovered.

Rabbits

Early studies with HIV infectivity of rabbits were discouraging, but more recent reports suggest that rabbits may be useful for the study of HIV infection (Kulaga et al., 1989; Filice et al., 1988). In early studies, six juvenile and twelve newborn rabbits inoculated with HIV showed neither seroconversion nor virus in their spleen cells or PMC (Morrow et al., 1987). In more recent experiments, rabbits in which aseptic thioglycollate peritonitis was induced were inoculated IP in the presence of IL-2 with H9 cells infected with HIV-1, or with cell-free supernatant of infected cells (Filice et al., 1988). HIV-1 antibodies were detected within two weeks by ELISA and HIV-1 antigens were detected in sera. All infected rabbits remained seropositive after one year and p24 protein was still present more than one year post-infection. Virus was isolatable by co-cultivation of PMC with uninfected H9 cells. Pooled PMC from five seropositive rabbits inoculated into a susceptible rabbit resulted in productive infection from which reisolations were made from PMCs up to seven months post-infection (Filice et al., 1988). Kulaga et al. (1989) have also shown that rabbits can be infected with HIV-1 by IV injection with a human T cell line infected with HIV-1. Rabbits with or without pre-infection with HTLV-1 were infected although rabbits pre-infected with HTLV-1 sero-converted more rapidly and gave stronger antibody responses than rabbits infected with HIV-1 only. No consistent signs of illness were observed, other than severe diarrhea in all of the animals and losses of body weight in some. One animal developed progressive mammary adenocarcinoma and two animals displayed transient posterior paralysis. Virus was isolated from blood and seroconversion was demonstrated (Kulaga et al., 1989).

Other non-primate animals

Horses were inoculated SC with clinical specimens and HIV-1 from cell culture (unpublished data). Transient antibody was seen in four of five and two of two respectively, but no other sign of infection was noted. Nine adult musk shrews (*Suncus murimus*) were inoculated IP with HIV-1. Neither seroconversion nor virus in their spleen cells or PMC were detected (Morrow et al., 1987).

Primates

Numerous experiments have been carried out on apes and new and old-world monkeys in the search for an animal that would duplicate the natural history of HIV infection in humans. To date, none has been found in which clinical symptoms are induced by injection of HIV-1 or HIV-1 infected tissue. Three squirrel, three Capuchin and two Spider monkeys and five marmosets were inoculated IP and intracerebrally (IC) with HIV-1 and four squirrel monkeys and four marmosets were similarly inoculated with clinical material (unpublished data). No viremia could be detected and no seroconversion occurred as measured by ELISA. IV administration of HIV-1 into four marmosets and four pygmy marmosets yielded transient symptoms of wasting and lymphoid hyperplasia. None of the animals seroconverted (by Western blot); however, two of the marmosets died 18 months after injection with signs of non-descript wasting and two pygmy marmosets died similarly at three and nine months (unpublished data).

Old-world monkeys reported to have been inoculated with HIV include the baboon, patas monkey, stumptail monkey, African green monkey, cynomolgus monkey, and rhesus monkey, using similar routes of administration and the same types of cultures and homogenates as described for early rodent studies (Morrow et al., 1987). No response was detected in any of the animals except that transient symptoms including weight loss, lymphadenopathy and diarrhea were reported in the rhesus monkeys, some of whom also showed transient antibodies to HIV.

Lowenstine et al. (1986) tested sera obtained from 526 old-world monkeys and apes of fifty species and twenty genera housed in zoos in the U.S. Initial screening was by ELISA, with follow-up testing of positive sera by Western blot. ELISA-negative cage mates of monkeys that tested positive also were followed by Western blot. Sero-reactivity to HIV-1 (probably represents SIV infection) was found in five species, all of African origin. All of the 23 (4%) monkeys positive for HIV-1 on Western blot had antibodies to the core proteins but only three had antibodies to the envelope (gp120).

In another study, five species of old-world monkeys were injected IC or IV with viral isolates or tissue suspensions of infected brain and viscera from AIDS patients (Wells et al., 1985; Yanagihara et al., 1987). Of the five species, only the rhesus monkey (*Macaca mulatta*) became infected and only after injection with viral isolates; four of ten adult and one of six infant monkeys developed a transient viremia and seroconverted as measured by radioimmunoprecipitation or ELISA.

However, zero of nine adult and zero of three infant rhesus monkeys seroconverted following inoculation with clinical specimens.

Although baboons have been reported to be unresponsive to infection with HIV-1, infection of baboons has been reported with HIV-2 (Letvin et al., 1988). Two macaques were also inoculated with HIV-2 in the same study. Preliminary studies showed that lymphocyte cultures from rhesus monkeys and baboons supported HIV-2 replication. The inoculum consisted of nonhuman primate peripheral blood lymphocytes stimulated with concanavalin A and exposed to HIV-2. The baboons were inoculated IV with 1 ml of the undiluted culture medium. Six weeks after injection, antibodies were detected in the baboons but not in the two *M. mulatta* inoculated at the same time. Virus was isolated from the baboons after three weeks and again from one of the baboons at four weeks postinoculation. The macaques remained negative for HIV-2 antibody throughout the follow-up period and the investigators were unable to isolate virus from macaque PMCs. The infection of baboons was of low titer and transient.

In another report (Dormont et al., 1989), ten rhesus monkeys were inoculated IC and IV with rhesus monkey cell adapted HIV-2 strains. Nine of ten inoculated monkeys showed seroconversion and in 7 of 10, HIV-2 replication was detectable by reverse transcriptase assay. One of the inoculated monkeys, in which both seroconversion and virus replication was demonstrated, began to lose weight 150 days after inoculation and subsequently developed other abnormal clinical and laboratory findings. With continued deterioration, this animal was killed at day 200 and necropsy results showed typical lesions of infection by actinomycetes of the meninges and the lungs; overall symptoms were not incompatible with an immunodeficiency-induced opportunistic infection.

Apes and AIDS

Until recently, the only animal in which a persistent viremia has been established with HIV-1 is the chimpanzee (Desrosiers and Letvin, 1987; Nara et al., 1987). Infection has been accomplished with tissue from AIDS patients, in vitro-infected autologous cells, blood products from other infected apes and with cell-free virus (Desrosiers and Letvin, 1987). Seroconversion has been observed consistently and HIV can be routinely recovered from peripheral blood specimens of the animals. After more than four years of observation, none of the animals has developed any AIDS-like symptoms (Fultz et al., 1989). Two chimpanzees experienced a transient generalized lymphadenopathy (Fultz et al., 1989), and infected juvenile chimps had an inhibited rate of weight gain (Fultz et al., 1986). No consistent changes have been reported in T4 lymphocytes, nor have any immunologic abormalities, opportunistic infections, tumors or death been seen.

The gibbon also has now been shown to be able to be clinically infected with HIV-1 (Lusso et al., 1988). Four gibbons, inoculated IV with approximately 10^6 50% tissue culture infectious doses of HIV-1 grown in H9 cells, were shown to develop specific humoral response, with antibodies toward HIV-1 envelope and core

proteins. Recovery of infectious virus (more than one year post-inoculation) from mitogen-stimulated PBM in all four animals was also reported.

Summary

The chimpanzee has been the only reliable, albeit imperfect, animal model of HIV-1 infection. Infection has been accomplished with tissue from AIDS patients, in vitro-infected autologous cells, blood products from other chimpanzees and cell free virus. Development of a persistent viremia and antibody production without other signs of infection has been a consistent observation. Infection of the baboon with HIV-2 needs further examination for its applicability to the needs for an animal model system. The recent demonstration that rabbits can be infected with HIV-1 and the utility of mouse-human chimeras for drug and vaccine development await further input from the scientific community. It is not unrealistic that in the near future other reliable animal models of HIV infection and AIDS disease might be forthcoming.

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