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Mini-Review

Animal models for anti-AIDS therapy

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Summary

Primate and non-primate species have been used to study the pathobiology of the simian immunodeficiency virus (SIV) and of the human immunodeficiency virus type 1 (HIV-1), respectively, and to develop new therapeutic regimes. Transgenic mice which express either the entire HIV-1 provirus or subgenomic fragments have been used to analyze viral gene products in vivo and may serve as models for the development of agents targeted to select viral functions. Chimeric mice which were created by transplanting human hematolymphoid cells into mice suffering from congenital severe combined immunodeficiency (*scid/scid* or so called SCID mice), can be infected with HIV-1 and allow one to study the entire HIV-1 replicative cycle. Type C murine leukemia virus models have been used to develop new prophylactic and therapeutic strategies but their use is restricted to the evaluation of select antiviral drug inhibition, targeted to retroviral genes common to both Lentivirinae and Oncovirinae. The role of various animal model systems in the development of anti-HIV-1 and anti-AIDS therapies is summarized.

Animal model; Antiviral therapy; Anti-AIDS therapy; Drug development; Transgenic mouse; Chimeric mouse

Introduction

Academic institutions as well as the pharmaceutical industry are investing significant resources to develop effective drug therapies which inhibit

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replication of the human immunodeficiency virus type 1 (HIV-1), the causative agent of the acquired immunodeficiency syndrome (AIDS) (Barre-Sinoussi et al., 1983; Popovic et al., 1984). Currently, only 3′-azido-3′-deoxythymidine (AZT, zidovudine) (Fischl et al., 1987; Richman et al., 1987; Volberding et al., 1990) and dideoxyinosine (ddI) (Editorial, Science, 1991) have been approved by the Food and Drug Administration (FDA) for the treatment of AIDS and related conditions.

Practical small animal models of HIV-1 viremia and disease would facilitate the selection of candidate anti-HIV-1 agents which may be considered for phase I clinical trials. A hypothetical ideal animal model for AIDS would be permissive for infection by HIV-1 and manifest an identical clinical spectrum of disease as that which occurs in human AIDS patients. Furthermore, the test animal would be relatively small to limit housing costs as well as to reduce the requirement for experimental drugs which may be limited and very costly. The lack of such an ideal animal model, however, has constrained research efforts aimed at understanding the pathobiology of HIV-1 and the development of antiretroviral therapy. Preclinical drug development, therefore, has made use of various surrogate animal retroviral model systems.

In general, animal retrovirus models can be used as models of viremia or disease (Ruprecht, 1989). Clinical modalities that can be evaluated in an animal model for viremia only include prevention of infection with vaccines or drug prophylaxis after de novo virus exposure, and treatment of chronic viremia. Surrogate models of HIV-1-induced disease may be used to evaluate antiretroviral agents which may have the capacity to restore immune function. Alternatively, animal models may be used to develop antimicrobial therapies for opportunistic infections or to evaluate chronic prophylactic treatment regimes. Moreover, animal models of Kaposi's sarcoma may be used eventually to assess cytotoxic therapy. Currently, model systems of HIV-1-induced neuronal disease, consisting of peripheral and central nervous system (CNS) damage, are not fully developed and therefore, insufficient information is available pertaining to therapeutic modalities for the treatment of CNS-related disorders.

HIV-1 infection of primates

Chimpanzees (Fultz et al., 1988) and gibbon apes were the first primates in which HIV-1 infection could be established following experimental injection. However, experimental establishment of immunodeficiency is problematic since HIV-1-positive chimpanzees which had been evaluated for up to 5 years have not developed AIDS. Some transient clinical signs of immunodeficiency have been observed, such as lymphadenopathy and a severe loss of $CD4^+$ lymphocytes ($>90\%$) as well as thrombocytopenia (Fultz et al., 1991). Since most HIV-1-infected chimpanzees have not developed signs of clinical disease or hematologic abnormalities, their virologic, serologic and other immune responses can be compared with those of asymptomatic HIV-1-infected individuals. This analysis may facilitate the identification of factors which

play a pivotal role in the development of disease. These animals could also be used to test chemotherapeutic agents which may alter the virus load or that enhance immune responses (Fultz et al., 1989). Even though the pathobiology of early HIV-1 infection in chimpanzees closely resembles that in humans, it is questionable as to whether a full spectrum of AIDS-related disease will develop in these primates. Therefore, the current HIV-1/chimpanzee model system can be used only to study viremia and its prevention or long-term therapy. However, lack of availability render these larger primates unsuitable for general antiviral drug studies.

Recently, eight pigtailed macaques (*Macaca nemestrina*) have been inoculated with HIV-1 and virus could be recovered from peripheral blood lymphocytes two weeks post-inoculation (Agy et al., Science 1992). Antibodies reactive against HIV-1 were detected 4 weeks post-inoculation. Anti-HIV-1 antibody titers remained elevated throughout the follow-up period which reached already 46 weeks. Virus could be isolated from peripheral blood leukocytes for up to 24 weeks post-inoculation. This early course of HIV-1 infection in *M. nemestrina* mimicks HIV-1 infection in humans. However, these monkeys will require further analysis to determine whether the macaques also develop AIDS-like disease. Even if no disease develops, HIV-1 infection of *M. nemestrina* may represent an excellent model system for viremia, and may be well suited for studying the effectiveness of antiviral drugs and vaccines.

HIV-2 and SIV infection of macaques

HIV-2 has been isolated and cloned from humans with acquired immunodeficiency and is able to replicate in macaques. However, not all inoculated monkeys become infected (Dormont et al., 1989). In viremic monkeys, only transient lymphadenopathy has been observed (Franchini et al., 1990). Currently, HIV-2 infection of macaques is in its developmental stages and is not sufficiently reproducible for drug testing (Castro et al., 1991; Stahl-Hennig et al., 1990). In contrast, infection of macaques with the simian immunodeficiency virus (SIV) (Letvin et al., 1985, 1987; Letvin and King, 1990), is considered by World Health Organization (WHO) experts to be an ideal animal model since SIV closely resembles HIV-1 in genomic organization (Chakrabarti et al., 1987; Franchini et al., 1987) and contains similar accessory genes (Park et al., 1991). The mutation rate, sexual transmission, control of replication and pathobiology are also quite similar to that of HIV-1 (Letvin et al., 1985; Ho et al., 1987; Gerber et al., 1991). In addition, several infectious molecular clones are available. Moreover, target cells and modes of transmission of SIV are very similar to those of HIV-1; SIV-infected monkeys develop CNS disease (Chakrabarti et al., 1991; Hurtrel et al., 1991) and mount an antiviral immune response very similar to that elicited by HIV-1 in humans. One exception is the maternal transmission of SIV, which pre- and intrapartum appears to be a relatively rare event, but may occur to a greater extent by breast-feeding (McClure et al., 1991).

SIV was originally isolated from captive rhesus macaques (Desrosiers et al., 1987) and has been isolated from other primate hosts such as mandrills and African green monkeys. These isolates vary markedly in their pathogenicity. Infection of primates with some strains results only in life-long asymptomatic viremia, while other isolates can induce immunodeficiency, CNS disease or an acutely fatal illness characterized by profuse watery diarrhea and weight loss. Even a given SIV isolate can differ in its pathogenicity, depending on the non-human primate injected; inoculation of some species results only in asymptomatic viremia. However, when macaques are inoculated with SIV strains isolated originally from macaques or mangabeys, persistent infection ensues which progresses over several months to immunodeficiency with a pathobiology similar to human AIDS. An interesting isolate is PBj14 (Fultz et al., 1989), an enterotropic and a highly lethal lentivirus. It was isolated from a pigtail macaque infected with an SIV_{smm} viral isolate, and an infectious, pathogenic molecular clone is now available. When injected into pigtail macaques, profuse watery diarrhea ensues approximately 5 days post-inoculation, and the animals die within two weeks. On pathological examination, marked lymphoid hyperplasia is seen, especially in the gastrointestinal tract, the spleen and lymph nodes. Interestingly, when monkeys are supported by intravenous hydration to treat their diarrhea, a few animals survive the initial acute illness to develop AIDS-like disease with a typical decrease in $CD4^+$ lymphocytes. Due to the striking similarities between SIV and HIV-1, results obtained with SIV/macaque model systems can be considered as the 'gold standard' in the development of drugs with activity against HIV-1 infection. For example, rhesus monkeys infected with SIV_{mac} and treated with 9-(2-phosphonylmethoxyethyl) adenine for 29 days were found to have a suppressed antibody response to gp120 which correlated to a reduction of viremia. Furthermore, no toxic side effects were noted during the treatment period (Balzarini et al., 1991).

HIV-1 infection of rabbits

Rabbits can be infected experimentally with HIV-1 following inoculation with either HIV-1-infected cells or concentrated virus stocks. Experimental injection of rabbits with HIV-1 has led to the detection of p24 antigen within 10–15 days, reaching peak values 6–9 weeks following infection. HIV-1 was isolated from peripheral blood mononuclear cells of infected rabbits 30, 60 and 180 days after infection (Filice et al., 1990). Unfortunately, however, frank disease resembling human AIDS has not been discovered to date, even though over 100 rabbits have been infected experimentally (Filice et al., 1988; Kulaga et al., 1989). The positive aspects of this animal model are the use of HIV-1 as the test virus and the availability of partially inbred strains of rabbits which require a relatively low maintenance cost. Furthermore, rabbit metabolism has been well characterized due to the extensive use of these animals in prior toxicological analyses, and the immunogenicity of various infectious agents,

TABLE 1

HIV-1 sequences in transgenic mice

HIV-1 sequence	Virus replication	Tissues with gene expression	Disease
Complete provirus	HIV-1 release only, no replication in mouse cells	tail, ears, skin and a few scattered cells in the liver and gastrointestinal tract	epidermal hyperplasia, pulmonary lymphoid infiltrates, lymphadenopathy, runting, death at 25 days
Defective HIV-1 provirus	none	skin, skeletal muscle, kidney, brain, eye, GI tract and spleen	renal disease, muscle wasting, runting, thymic atrophy, papillomatous skin lesions
HIV-1 LTR-*tat*-3	none	skin, liver in 1/3 transgenic lines	dermal lesions in transgenic male mice resembling Kaposi's sarcoma, hepatic neoplasms
HIV-1 LTR linked to reporter gene:			
HIV-1 LTR linked to CAT gene	none	thymus, tail, eye, heart, spleen. Among monocyte-macrophage derived cells, maximal activity in Langerhans cells	none
HIV-1 LTR linked to CAT gene	none	glial cells of cerebral cortex, basal ganglia and cerebellum	none
HIV-1 LTR linked to CAT gene	none	thymus, spleen and spinal cord; after morphine administration, high CAT expression in brain (hypothalamus, midbrain), spinal cord and eyes	none
Strain a: HIV-1 LTR linked to CAT Strain b: murine α-crystalline A transcriptional control elements fused to HIV-1 *tat*	none	strain a × strain b: CAT transactivation in the eyes of F_1 offspring carrying both transgenes none	none
HIV-1 LTR controlling firefly luciferase or β-galactosidase genes	none	skin; activation of HIV-1 LTR in epidermis upon exposure to UV, psoralen and sunlight	none
HIV-1 LTR controlling SV40 T and t antigen genes	none	thymus, lymph nodes, spleen and skin	respiratory insufficiency secondary to thymic hyperplasia

See text for references.

including HIV-1 subunits, has been evaluated in rabbits. The relative disadvantages to the use of rabbits is the very high inoculum of HIV-1 required to establish viremia. Moreover, rabbit immunology has not been characterized thoroughly, and no AIDS-like pathology has been reported in HIV-1-infected rabbits. Currently therefore, HIV-1 infection of rabbits is considered experimental and is generally not applicable for antiviral drug development.

Murine transgenic systems

Transgenic mice have been created harboring complete HIV-1 proviral sequences (Leonard et al., 1988), subgenomic fragments (Dickie et al., 1991; Vogel et al., 1988; Khillan et al., 1988) or reporter genes linked to the HIV-1 LTR (Khillan et al., 1988; Leonard et al., 1989; Harlan et al., 1991; Prakash et al., 1990; Morrey et al., 1991; Skowronski et al., 1991) (see Table 1). An important caveat is that HIV-1 cannot replicate in mice because: (1) mice do not have CD4 receptors and (2) even if mouse cells are transfected with human CD4 receptor cDNA and express adequate levels of this gene product, there is still a block to HIV-1 infection, presumably at a postreceptor level (Adachi et al., 1986; Chesebro et al., 1990).

1.a. Transgenic mice carrying full-length HIV-1 transgenes

Leonard et al. (1988) created transgenic mice which contain the complete HIV-1 provirus. HIV-1 expression or signs of disease were not detected in any founder animals, even though integrated proviral copies were demonstrated. One founder female mated to a normal syngeneic male produced F_1 litters that survived approximately 25 days postnatally. These F_1 animals had stunted growth, perivascular pulmonary lymphocytic infiltrates, epidermal hyperplasia involving tail, ears, nose and feet and were found to have splenomegaly, lymphadenopathy and involution of the thymus. Selective destruction of $CD4^+$ lymphocytes, the characteristic manifestation of human AIDS, was not observed in F_1 mice. HIV-1 virions could be isolated from affected F_1 mice only and were able to infect human $CD4^+$ cells but not murine fibroblasts. Furthermore, affected F_1 mice were unable to support replication of HIV-1, even though virus release was demonstrated.

The relevance of these HIV-1 transgenic mice to human HIV-1 infection and AIDS is unclear. First, only late steps of the viral replication cycle occur since virions released into the bloodstream were unable to infect non-permissive murine target cells. Secondly, it is debatable as to whether the severely affected F_1 mice exhibited lesions characteristic of human AIDS. The skin disease of these animals does not resemble the skin lesions which occur in AIDS patients, such as seborrheic-like dermatitis, psoriasis or ichthyosis (Soeprono et al., 1986; Goodman et al., 1987; Matis et al., 1987). However, the pulmonary interstitial lymphoid infiltrates resemble the non-specific interstitial pneumonitis of adult AIDS patients (Suffredini et al., 1987). The lethal congenital

disease of these F_1 transgenic mice may have been due to the expression of HIV-1 gene products since the HIV-1 LTR is transcriptionally active in some of the tissues affected. Alternatively, this F_1 phenotype may have arisen from insertional mutagenesis by the integration of the transgene into the genome with subsequent disruption of important murine genes, similar to the transgenic mice described by Jaenisch (1988). In any event, shortly after the publication of this model system, all transgenic mice perished in a laboratory accident (Ezzell, 1988).

1.b. Transgenic mice carrying defective HIV-1 proviruses

Three lines of transgenic mice were created which carry an HIV-1 provirus containing an in-frame deletion of *gag* and *pol* sequences (Dickie et al., 1991). The defective provirus encodes the complete sequences for *env*, *tat*, *rev*, *nef*, *vpu* and *vif* as well as a p17/p34 fusion gene product. F_1 offspring had a high mortality rate possibly due to renal disease characterized by proteinuria and interstitial nephritis. Renal tissue had ultrastructural features consistent with glomerulosclerosis, with HIV-1 proteins present in the glomeruli of affected mice. Several other tissues contained HIV-1-specific mRNA and expressed viral-proteins to various degrees (Dickie et al., 1991).

1.c. Transgenic mice expressing HIV tat.

Vogel et al. (1988) created transgenic mice containing the HIV-1 *tat* gene linked to the HIV-1 LTR. Skin lesions consisting of spindle cell proliferation developed and tumors formed in male mice while female mice failed to develop skin lesions or tumors, even though they synthesized similar levels of *tat* mRNA. Furthermore, there was a higher incidence of hepatocarcinoma among male mice. Development of tumors was apparently influenced indirectly by *tat*-mediated growth factor release from the skin (Vogel et al., 1991). These transgenic mice have also been used to study UV activation of the HIV-1 LTR. Exposure of mice to UV light led to the expression of *tat* almost exclusively in the epidermal portion of the skin (Vogel et al., 1992).

Transgenic mice carrying the chloramphenicol acetyltransferase (CAT) gene under the transcriptional control of the HIV-1 LTR were created by Khillan et al. (1988). These mice were mated with transgenic animals carrying the HIV-1 *tat* gene linked to a mouse α-crystalline A transcriptional control element. Transactivation of the HIV-1 LTR occurred only in the eye of F_1 offspring, the tissue in which the α-crystalline A promoter/enhancer element exhibits preferential activity. These data demonstrate that HIV-1 *tat* is functional in mice.

1.d. Transgenic mice carrying the HIV-1 LTR linked to reporter genes

Several laboratories have shown that the HIV-1 LTR is transcriptionally active in transgenic mice. Four lines of transgenic mice containing the CAT gene fused to the HIV-1 LTR were created by Leonard et al. (1989). Tissue-specific CAT expression was detected in the eye, heart, spleen, thymus and tail of all transgenic animals. Circulating lymphocytes and monocytes expressed

TABLE 2

Chimeric mouse model systems

Model feature	SCID-hu	hu-PBL-SCID	HID-*bg/nu/xid*
Reconstitution	SCID-hu Thy−: transplantation with human fetal thymus under the renal capsule SCID-hu Thy/Liv: transplantation with human fetal thymus and liver SCID-hu LN: transplantation of human lymph nodes into the mammary fat pads SCID-hu Bone: subcutaneous insertion of human bone	Adult human PBL, from EBV^+ of EBV^-, and CMV^- as well as HBV^- donors	Adult human bone marrow cell transplants
Surviving human cell populations	proliferation of multilineage hematopoietic cells, differentiation of human T- and B cells phenotypically and functionally normal mature human lymphocytes	$CD4^+$ and $CD8^+$ human T-cells, B-cells and to a lesser degree macrophages phenotypically and functionally normal mature human lymphocytes	Human macrophage progenitors
Maximal length of graft survival	SCID-hu Thy/−: 3 months SCID-hu Thy/Liv: > 12 months SCID-hu LN: 3 months SCID-hu Bone: 4–5 months	22–48 weeks as measured by human IgG production	Up to 5 weeks
Humoral immunity	production of human IgG, primary and secondary responses	production of human IgG, secondary responses to tetanus toxoid	Not reported
Cellular immunity	normal appearing lymphoid follicles, T-cell compartment functionally intact including primary responses	secondary responses; T-cell proliferation to mitogen and alloantigens detectable up to 6 weeks	Not reported
HIV-1 isolates capable of replication	only primary patient isolates but not laboratory adapted strains	many laboratory strain (IIIB, MN, RF, SF2, SF13, SF33, DFCI-HT1), primary patient isolates and AZT-resistant virus	Not reported
Route of HIV-1 inoculation	SCID-hu Thy/−: intrathymic injection SCID-hu LN: i.v.	i.p.	Not reported

*For references please see Bosma et al., 1987; Custer et al., 1985; Schuler et al., 1986; Mąlynn et al., 1988; McCune et al., 1988, 1991; Peault et al., 1991; Namikawa et al., 1990; Krowka et al., 1991; Kaneshima et al., 1991; Kyoizumi et al., in press; Vanderkerchove et al., 1991; Mosier et al., 1991; Mosier, 1991; Kamel-Reid and Dick, 1988.

CAT activity when co-cultivated in the presence of mitogens or various cytokines. The highest levels of CAT activity were detected in Langerhans cells of the skin.

Two additional reports were published describing transgenic mice carrying the HIV-1 LTR linked to the CAT gene. Transgenic mice were generated by Harlan et al. (1991) to assess the relative transcriptional activity of the HIV-1 LTR in brain tissue by utilizing immunocytochemical staining techniques. Cells with morphological features of astrocytes or microglia were labeled in the cerebral cortex, basal ganglia and the cerebellum. Prakash et al. (1990) developed transgenic mice carrying the HIV-1 LTR linked to the CAT gene and demonstrated that the transgene was expressed in thymus, spleen and spinal cord. Following morphine administration, high CAT expression was detected in the brain, especially in the hypothalamus and mid-brain, the spinal cord and the eyes. Mice containing the HIV-1 LTR linked to either the firefly luciferase or the β-galactosidase reporter gene were constructed by Morrey et al. (1991). The HIV-1 LTR was activated in vivo by exposure to sunlight or ultraviolet (UV) radiation. The photosensitizing agent, psoralen, when topically applied to the skin of these mice, shortened the time required for UV activation of the LTR.

Three lines of transgenic mice were generated by Skowronski et al. (1991) which carried the HIV-1 LTR linked to the SV40 T and t-antigen genes. Enlargement of the thymus was the only consistent pathological finding. In all transgenic lines, the transgene was transcriptionally active in the skin, thymus, spleen and lymph nodes. The highest levels of T- and t-antigen transcripts were found in B-cells, while total T-cells and T-cell sub-populations contained 5 to 10-fold fewer transcripts.

The pattern of constitutive expression of genes controlled by the HIV-1 LTR in transgenic mice is thought to arise from the interaction of the LTR with cellular transcription factors. Such transgenic mice may prove useful for the analysis of tissue-specific transcription factors, environmental factors or pharmacological agents acting through the HIV-1 LTR either as potentiators or inhibitors of gene expression. Candidate antiviral agents may be administered to transgenic mice which contain the HIV-1 LTR linked to a reporter gene to determine an agent's potential to activate or inhibit the function of the HIV-1 LTR in vivo.

The SCID mouse system

SCID mice, derived from the C.B-17 strain, carry an autosomal recessive mutation at the severe combined immunodeficiency *scid* locus (Bosma et al., 1987; Custer et al., 1985). Homozygous *scid/scid*, so-called SCID mice, lack functional T- and B-cells due to a defective VDJ recombinase mechanism (Schuler et al., 1986; Malynn et al., 1988). This mutation leads to a congenital loss of B- and T-cell maturation. Several experimental systems have been developed by engraftment of SCID mice with either fetal lymphoid organs or

peripheral blood leukocytes from normal adult donors (see Table 2).

SCID-hu mice

Human/mouse chimerae have been created by transplanting human fetal liver, thymus, lymph nodes or bone tissue into SCID mice (McCune et al., 1988; Peault et al., 1991; Namikawa et al., 1990; Krowka et al., 1991; Kaneshima et al., 1991; Kyoizumi et al., in press; Vanderkerckhove et al., 1991; McCune et al., 1991). Human tissues were transplanted under the renal capsule (thymus and liver), into the mammary fat pads (lymph nodes) and subcutaneously (bone fragments) (Kyoizumi et al., in press). The four different types of chimerae created are categorized according to the type and combination of tissue transplanted. Different lineages of human hematopoietic cells were able to differentiate and proliferate. Phenotypically and functionally normal mature human T- and B-lymphocytes were observed. SCID-hu mice transplanted with fetal thymus, liver and lymph nodes synthesized human IgG. Typical primary and secondary antibody response occurred after antigen challenge. Furthermore, lymph nodes contained normal appearing lymphoid follicles. Both primary and secondary T-cell responses can be obtained.

SCID-hu mice are susceptible to HIV-1 infection. The most commonly used chimerae for HIV-1 studies are SCID-hu Thy/- and SCID-hu LN mice (Kaneshima et al., 1991). However, laboratory-adapted strains of HIV-1 do not replicate in SCID-hu mice. The efficacy of antiviral therapy has been evaluated in HIV-1-infected SCID-hu mice. For example, post-exposure prophylaxis with AZT was found to prevent viremia (Shih et al., 1991). Antiviral agents can be assessed easily in vivo since the efficacy of single-agent or drug combinations can be determined by measuring the fraction of viremic animals after a 2-week course of drug therapy.

hu-PBL-SCID mice

Mosier et al. (1988) used an alternate approach to create human/mouse chimerae. Human peripheral blood leukocytes were injected into the peritoneal cavity of SCID mice which led to the stable engraftment of a functional human immune system that survived 22–48 weeks as measured by the production of human IgG.

Phenotypically and functionally normal human lymphocytes could be isolated from the blood and lymphoid tissues of the chimerae. hu-PBL-SCID mice can be infected with HIV-1 reproducibly by intraperitoneal injection. These animals support not only the replication of primary patient isolates but also that of laboratory-adapted strains such as III-B, MN, RF, SF2, SF13, SF33, DFCI-HT1, and AZT-resistant virus (Mosier et al., 1991; Mosier, 1991; Mosier, personal communication).

Chimerae of the bg/nu/xid genotype

Mice with the triple mutation *bg/nu/xid* were given normal human bone marrow cell transplants following irradiation (Kamel-Reid and Dick, 1988).

The *nu* mutation leads to a non-functional thymus, while the *bg* mutation dramatically reduces the number of natural killer cells, and the *xid* mutation leads to a low level of lymphokine-activated killer cells. Following transplantation, human macrophage progenitor cells were found to differentiate and survive for up to 5 weeks. The susceptibility of human macrophages to HIV-1 infection in vivo may be investigated with these chimerae; however, to date there are no data available pertaining to HIV-1 infection in these mice.

HIV-1 infection of mice

A persistent HIV-1 infection of normal Swiss mice has been accomplished by a single intraperitoneal injection of U937 cells producing high titers of HIV-1 virus (Locardi et al., 1992). Antibodies to HIV-1 could be detected more than 500 days after the original infection, and p24 antigen was present in approximately 50% of the infected mice. Residual human DNA could not be detected from peritoneal cells or peripheral blood mononuclear cells, indicating that viremia was a consequence of infected murine cells. This mouse model system may prove useful as a means to assess the efficacy of antiretroviral agents. However, HIV infection of mice is currently in its experimental stage, since additional evidence relating to the mechanism of infection and establishment of viremia awaits future studies.

Non-primate Lentivirus/animal models: large animal models

Bovine immunodeficiency virus (BIV) infection of cattle, caprine arthritis-encephalitis virus (CAEV) infection of goats, visna virus infection of sheep and equine infectious anemia virus (EIAV) infection of horses represent lentiviral systems with interesting pathobiology. Van der Maaten et al. (1972) reported the isolation of BIV from cattle with persistent lymphocytosis. The cattle were found to have lymphoid hyperplasia and mild lymphocytic perivascular cuffing in the brain. Subsequent studies have shown the virus to be antigenically and genetically related to other lentiviruses (Gonda et al., 1987). Furthermore, BIV has been shown to encode regulatory proteins analogous to those of HIV-1 (Oberste et al., 1991; Pallansch et al., 1992). Experimental infection of calves with BIV leads to mild lymphoproliferative alterations within the first 6 weeks following infection. The early pathological changes are follicular hyperplasia in lymphnodes and spleen as well as increased numbers of lymphocytes in peripheral blood (Carpenter et al., 1992). These changes are characteristic of several immunosuppressive lentiviruses, including HIV and SIV and suggest that BIV infection of calves may be a useful animal model for the study of the early events in HIV pathogenesis. However, additional studies are needed to define the long-term pathogenic effects of BIV infection in vivo in order to determine the appropriateness of BIV as an animal model of the HIV-associated immunodeficiency syndrome.

CAEV infection of goats causes an acute leukoencephalitis. Persistent infections give rise to a chronic arthritis due to lymphocyte infiltration and

proliferation of synovial cells (Crawford et al., 1980).

Visna and maedi virus infection of sheep cause prominent neurologic and pulmonary symptoms. Natural and experimental infection of sheep leads to replication of visna-maedi at the site of entry (Nathanson et al., 1985). Sheep typically develop viremia whereby infection spreads to other organ systems, including the lungs and CNS, which eventually leads to partial paralysis, weight loss and shortness of breath (Haase, 1986). Naturally infected sheep generally become symptomatic during the second year of infection and die following a protracted and progressive illness.

Infection of horses with EIAV leads to recurring cycles of virus replication, plasma viremia, hemolytic anemia, edema, leukopenia and thrombocytopenia (Issel and Coggins, 1979). Viremia and disease appear at irregular intervals during the first year of infection which has been attributed to the generation of antigenically distinct viral isolates (Payne et al., 1987; Payne, 1984; Salinovich et al., 1986). The disease is typically suppressed by an active immunological response which leads to a latent viral infection (Montelaro et al., 1984). The principal neutralizing domain of the surface unit glycoprotein gp90 has been discovered and found to be similar to the V3 loop of HIV-1 (Ball et al., 1992). This analysis may provide an important foundation for further analyzing the protective immune response generated during persistent EIAV infections.

Because of the large size of the animal hosts and the relative lack of knowledge regarding their genetics, immunology and metabolism, these lentiviral models do not seem practical for drug development.

Non-primate lentivirus/animal models: FIV infection of cats

The feline immunodeficiency virus (FIV) is a lentivirus that was first isolated from a group of cats in Petaluma, California (Pedersen et al., 1989). FIV has been molecularly cloned and contains all the typical lentiviral regulatory genes. The genome of FIV shares 30–50% homology with HIV-1 and encodes a reverse transcriptase enzyme that is inhibited by the same antiviral drugs as HIV-1. Pathogen-free cats have been inoculated with FIV and develop immunodeficiency similar to HIV-1 infection in humans or SIV infection in rhesus monkeys. Infected cats develop a transient primary disease 4–8 weeks following virus inoculation which continues for several weeks. This primary phase is associated with fever, leukopenia, lymphadenopathy and gastrointestinal illness similar to the mononucleosis-like primary complications of HIV-1 infection. Once cats recover from the primary phase of infection, an asymptomatic period ensues lasting several weeks, months or years, depending on the virus strain. Infection then proceeds which gives rise to altered $CD4^+/CD8^+$ ratios which have been correlated with seropositivity (Ackley et al., 1990a, 1990b; Hoffman-Fezer et al., 1992). Experimentally infected pathogen-free cats, however, have a less pronounced alteration of T-cell subsets compared with naturally infected cats, presumably due to housing in pathogen-free facilities. Recently, an FIV strain designated NCSV-1 has been

isolated which gives rise to a decline in $CD4^+/CD8^+$ ratios within 4–6 weeks post-inoculation. Signs of disease, such as weight loss, ocular pathology, gingivitis and respiratory infections appear within 18 months in a small percentage of random source specific pathogen-free cats (Tompkins et al., 1991 and personal communication). Moreover, FIV infection in naturally and experimentally infected cats leads to CNS involvement with brain lesions consisting of perivascular mononuclear cell infiltrates, glial nodules and diffuse gliosis observed in the midbrain and thalamus. Virus has been isolated from several brain regions including the cerebral cortex, caudate nucleus, midbrain, cerebellum and brainstem. FIV infection of the CNS may therefore serve as a useful model of HIV-1 infection of the human CNS (Dow et al., 1990). The relative disadvantages of this model include an incomplete knowledge of feline immunology and hematopoiesis as well as a lack of inbred strains. The strong points of this model system include the use of an immunosuppressive lentivirus, a relatively small animal host, and the possibility of carrying out field trials, particularly if vaccine development is considered.

Oncornavirus retrovirus/animal models

Type C retroviruses, members of the Oncovirinae sub-family of retroviruses, differ significantly in their molecular structure from lentiviruses. They encode only the universal retroviral genes *gag*, protease, reverse transcriptase, ribonuclease H, integrase, envelope and lack the regulatory genes typical of lentiviruses. Furthermore, these retroviruses do not require CD4 receptor molecules for infection. For drug development, the feline leukemia virus (FeLV), and various murine leukemia virus (MuLV) systems have been used.

FeLV infection of cats

Feline leukemia virus (FeLV), in contrast to FIV, is a Type C virus that causes a wide spectrum of disease in cats including bone marrow suppression, immunodeficiency and leukemia. The virus replicates to very high titers in bone marrow. High titers of FeLV are found in saliva, and the transmission of FeLV occurs primarily by the oral-nasal route or by biting (Hoover et al., 1989; Tavares et al., 1989). The relative disadvantages of FeLV in addition to its non-lentiviral nature are the lack of inbred cats and a paucity of information pertaining to feline immunodeficiency. Furthermore, few immunological reagents are available and few lymphokines are known in this system. The pathogenesis of FeLV-induced immunosuppression is not understood thoroughly and may differ significantly from that of HIV-1-induced AIDS. For this reason, the FeLV/cat system may only be applicable as a model for viremia and not for HIV-1 disease. If immunomodulators are evaluated in vivo in FeLV-infected cats, it is important to consider the potential differences in immunopathogenesis between HIV-1 and FeLV, and to interpret animal data with caution.

MuLV systems

Various new therapeutic modalities were developed in MuLV models: post-exposure prophylaxis, therapy of chronic viremia, transplacental therapy with drugs and antisera, prevention of milk-borne maternal transmission of virus and the quantitative analysis of combination regimens involving AZT and interferon-α (Ruprecht et al., 1990a). Also, therapy of neurotropic retrovirus infections was first evaluated with murine leukemia viruses (Sharpe et al., 1987). The first effective retroviral vaccine was found in 1959 against Friend MuLV (Friend, 1959). The various MuLV systems employed in drug development are discussed below.

LPBM-5

The LPBM-5 virus was isolated from the bone marrow of C57BL/6 mice infected with the radiation induced Duplan-Laterjet strain of MuLV (Laterjet and Duplan, 1962). LPBM-5 is a complex of murine leukemia viruses consisting of mink-cell focus forming viruses (MCF) and replication-competent, ecotropic viruses which are non-pathogenic. The third component consists of replication-defective virus which causes an acquired immunodeficiency called murine AIDS (MAIDS) (Aziz et al., 1989). It is important to keep in mind, though, that MAIDS results from infection with a Type C retrovirus and not a lentivirus.

LPBM-5 infection leads to polyclonal B-cell proliferation, hypergammaglobulinemia and loss of T-cell function. Infected animals develop lymphadenopathy, splenomegaly and eventually die approximately 26 weeks post-inoculation of B-cell lymphomas or opportunistic infections (Mosier et al., 1985; Mosier et al., 1986). Therefore, the terminal stage of disease and subclinical features are reminiscent of human AIDS. Late in the course of disease, oligoclonal B-cell proliferation may result in frank malignant lymphoma. Development of immunodeficiency by LPBM-5 is associated with the replication-defective viral genomes and is dependent upon the presence of normal or elevated circulating levels of functional $CD4^+$ T lymphocytes.

Recently, the pathogenesis of LPBM-5-induced immunodeficiency has been linked to the expression of an abnormal *gag* precursor, Pr60*gag*. This abnormal virus gene product is expressed on infected B-cells and appears to interact with T-cells by a mechanism consistent with that of a super antigen (Hügin et al., 1991). A massive stimulation of $CD4^+$ T cells ensues with the secretion of cytokines. A positive feedback loop is established since the excreted cytokines recruit additional B-cell targets for virus replication which leads to increased expression of the abnormal *gag* gene product. Infection of susceptible mice with LP-BM5 has been used to study the efficacy of antiviral drugs as well as immunomodulating agents. AZT therapy (Basham et al., 1990; Pornoi et al., 1990; Ohnota et al., 1990; Eiseman et al., 1991) has been shown to block partially LP-BM5 infection, in contrast to effective AZT post-exposure

prophylaxis in RLV-inoculated mice (Ruprecht et al., 1986). However, continuous oral AZT therapy of C57BL/6 mice given a low dose of virus prevented LP-BM5 immunodeficiency (Ohnota et al., 1990; Eiseman et al., 1991). Therapy with the immunomodulators diethyldihydrocarbonate (DTC) (Hersh et al., 1991) and cyclosporin A (Cerny et al., 1991) both suppressed the course of LP-BM5-induced disease. It is uncertain whether LPBM-5 infection of mice will be a relevant model for HIV-1-induced disease; it is not known whether the two viruses cause disease by common mechanisms. Moreover, in contrast to HIV-1, LPBM-5 does not infect $CD4^+$ T-cells which suggests that this virus infection would differ in pathobiology, even though the ultimate outcome is acquired immunodeficiency. Immunostimulatory agents or immunomodulators that are considered for evaluation in vivo would have to be analyzed with great care due to the possible difference in pathobiology between LPBM-5 and HIV-1.

Cas-Br-E

The Cas-Br-E virus was isolated from the brain of a paralyzed wild mouse that was trapped in the Lake Casitas area of California (Gardner et al., 1973a,b). Cas-Br-E is an ecotropic, N-tropic virus for which a virus titration assay is available in the form of the XC plaque assay. Cas-Br-E has been cloned by Jolicoeur et al. (1984). By mutational analysis, the potential to induce the typical hind limb paralysis was found to be associated with sequences within the gene encoding the envelope glycoprotein gp70 (DesGoseillers et al., 1985). Neonatal inoculation of susceptible laboratory mice leads to 100% hind limb paralysis within 3–5 months, while inoculation of adult mice does not lead to the development of disease. Neonatally infected mice develop immune tolerance which is a necessary prerequisite to support viral replication and eventual development of disease. Cas-Br-E was the first retroviral disease demonstrated to be sexually transmitted (Gardner, 1990). However, in nature, milk is the major route of infection. The initial target cell of the virus is probably a lymphoid cell since some animal strains do not develop neurological disease but rather develop either T-cell or B-cell lymphomas. Initially, the virus replicates in the spleen, and the CNS becomes infected subsequently. Neuropathological examination of paralyzed mice has demonstrated polioencephalomyelopathy in the anterior horns of the spinal cord, featuring a non-inflammatory spongiform alteration. The dentate nucleus of the cerebellum and the brainstem are also affected (Oldstone et al., 1977; Oldstone et al., 1980; Brooks et al., 1980). Cas-Br-E-induced spinal cord disease involves white matter to a lesser degree than HIV-1 induced myelopathies, and the pathogenesis remains uncertain (Petito et al., 1985). The reproducibility of infection and relatively short latent period of neurologic disease induced by Cas-Br-E in newborn susceptible FV- 1^n mice render this model suitable for testing transplacental therapy and effectiveness of antiretroviral agents targeted to the CNS (Sharpe et al., 1987).

TABLE 3

Relative effectiveness of candidate antiviral agents in the RLV model

Therapy	Optimal dose schedule	Optimal inhibition of splenomegaly	Observations in mice	Clinical experience in humans
AZT combined with interferon-α (rHuIFN-αA/D)	15 mg/kg/d p.o. 5×10^5 U/kg i.p., qd	97%	combination is highly synergistic in vivo and nontoxic. 100% effective in preventing viremia when given as post-exposure prophylaxis	ACTG trial 068 on-going; combination therapy against KS safe and effective
AZT	165 mg/kg/d p.o.	98%	anemia and leukopenia developed in some mice. Effective as postexposure chemoprophylaxis and for treatment of chronic viremia	effective in humans with HIV-1 infection; FDA-approved. Dose range in mice is comparable to human doses.
Interferon-α	5×10^5 U/kg i.p., bid	94%	linear dose–response	used in KS patients (reviewed in)
Castanospermine	300 mg/kg/d p.o.	79%	therapeutic window appears relatively narrow	trials with another inhibitor of glycosylation are ongoing; some toxicity was seen
6-O-butanoyl-castanospermine	346 mg/kg/d p.o.	55%	therapeutic window appears relatively narrow	
Suramin	40 mg/kg/d i.v., q3d	56%	therapeutic window appears relatively narrow	too toxic and ineffective in AIDS patients

K.S.: Kaposi's sarcoma; p.o., oral; i.p., intraperitoneal, qd, daily; bid, twice daily; i.v., intravenous; q3d, every third day.
Please see text for references.

Rauscher murine leukemia virus complex

Rauscher murine leukemia virus (RLV) (Rauscher, 1962) is a complex which consists of a replication-competent helper virus that causes B-cell lymphomas, and a replication-defective spleen focus-forming virus (SFFV) which is responsible for erythroid disease (Weiss et al., 1985). The pathobiology of Rauscher-induced disease is nearly identical to the disease caused by the Friend virus anemia strain. The genomes of Friend and Rauscher viruses share 90% nucleic acid identity which suggests that they are derived from a common murine leukemia virus (Bestwick et al., 1984). Adult mice are susceptible to infection by RLV and FLV, in contrast to most other MuLV viral isolates. Eight days post-inoculation, spleen colonies develop, each representing a successful virus infection; the disease, therefore, is polyclonal. Two weeks post-inoculation, spleens are palpably enlarged, and within approximately 4–6 weeks, the animals die of erythroleukemia (Weiss et al., 1985). During early stages of splenomegaly, cellular transformation does not occur, and consequently it is not possible to establish cell lines from spleens during this period of infection. However, later in the leukemic phase it is quite easy to establish permanent stable cell lines from the spleens of these animals. Rauscher and Friend virus infection of susceptible mice have been used extensively for drug development since the number of spleen colonies which develop (ca. 8 days post-infection) and the degree of splenomegaly (2–3 weeks post-infection) are proportional to the virus titer and hence can be related to the efficacy of anti-retroviral agents (Chirigos, 1964).

Inhibition of RLV-induced splenomegaly has been used to evaluate a number of antiretroviral agents including AZT and interferon-α (Ruprecht et al., 1988, 1990a,b). In Table 3, the agents are listed in order of their therapeutic efficacy. A 20-day treatment regime consisting of AZT alone or in combination with recombinant interferon-α completely inhibited splenomegaly and viremia. However, although both regimens were equally active, AZT as a single agent was more toxic. An effective dose–response to inhibition of splenomegaly by administration of recombinant interferon-α was discovered in RLV-infected mice. Inhibitors of glycosylation such as castanospermine (Ruprecht et al., 1989) and its 6-O-butanoyl analog (Ruprecht et al., 1991) showed partial effectiveness in terms of inhibition of splenomegaly, but the agents were clearly more toxic than AZT even in the dose ranges that were tolerated. Now that results of clinical trials in patients infected with HIV-1 are becoming available, it is noteworthy that this simple Type C murine retrovirus system could predict the relative efficacy of antiretroviral treatment regimens. Suramin, the first candidate drug tested in AIDS patients, when administered to RLV-infected mice, led to a partial inhibition of splenomegaly only (Ruprecht et al., 1985). When administered to AIDS patients, suramin was found to have no significant effect upon clinical improvement or lowering circulating levels of p24 antigen (Eeftink Schattenkerk et al., 1988). While castanospermine which was moderately active in the RLV system (Ruprecht et al., 1989) has not been

tested in AIDS patients per se, another inhibitor of glycosylation, *N*-butyl-deoxynojirimycin, reportedly had side effects and is considered only for combination regimen (AIDS/HIV Treatment Directory, 1991). In general, the RLV model identified treatment regimens with clinical benefits in HIV-1-infected humans, such as AZT (Fischl et al., 1987; Richman et al., 1987; Volberding et al., 1990), interferon-α, and AZT combined with recombinant interferon-α (Mildvan et al., 1991; Kovacs et al., 1989; Krown et al., 1990; Fischl et al., 1991; Fischl, 1991).

Friend murine leukemia virus

Friend virus (FV) is comprised of a replication-competent helper virus and various strains of replication-defective spleen focus-forming viruses (SFFV), causing either polycythemia ($SFFV_p$) or anemia ($SFFV_a$) (Weiss et al., 1985). FV has been shown to infect macrophage precursors (Marcelletti and Furmanski, 1979), but is considered to be predominantly B-cell tropic (Rosen et al., 1985). The early events following FV + $SFFV_a$ infection are the development of erythroid proliferation whereby the liver and spleen become infiltrated with immature erythroblasts culminating in hepatosplenomegaly and severe anemia, while the thymus and lymph nodes typically remain unaffected. During later stages of infection, the animals develop frank leukemia and succumb within 8–12 weeks. The oncogenic potential of the FV complex is associated with the truncated envelope gene (gp55) of the defective SFFV component (Gliniak and Kabat, 1989). Neonatal as well as adult mice are susceptible to the disease. FV infection of (B10.A × A/WySr) F1 mice (Morrey et al., 1990) exhibits some signs of HIV-1 infection in man, including immune suppression with a concomitant development of antiviral antibodies and the continued presence of low levels of infectious virus. Splenic helper and suppressor/cytotoxic T-cell functions are also impaired, with a low CD4/CD8 ratio and a depressed response to phytohemagglutinin (PHA). FV infection has been used as a model system to evaluate candidate antiviral and immunomodulatory therapies. For example, mice have been treated with AZT following FV inoculation. Treated mice had a marked inhibition of splenomegaly, elimination of infectious centers and circulating FV. Although treatment with AZT inhibited FV-induced splenomegaly, prevented depression of total T-cells and increased survival, low levels of virus could still be detected in some mice which may be latent or below a critical threshold to cause disease (Morrey et al., 1991). The course of FV infection can also be altered by immunomodulatory agents which affect T- and B-cell function. Imexon administration has been shown to improve T-cell function as measured by PHA-induced blastogenesis while reducing splenomegaly and viral titers (Morrey et al., in press). However, data on immunotherapy generated in the FV system should be interpreted cautiously as far as implications for therapy of human HIV-1 infection is concerned because the pathobiology of FV and HIV-1 is likely to differ significantly.

Moloney murine leukemia virus

Moloney murine leukemia virus (MoMuLV) is a T-cell tropic virus that causes disease in neonatally inoculated animals with a latent period of 3–6 months prior to the development of T-cell leukemia/lymphoma (Weiss et al., 1985). Transgenic mice which harbor a single proviral copy of MoMuLV (Mov mice) at unique chromosomal sites have been developed and used to evaluate transplacental and neonatal antiviral therapy (Sharpe et al., 1988; Lee et al., 1991). More than 40 Mov strains were generated by exposing mouse embryos at various stages of development to MoMuLV (Jaenisch, 1976; Jahner and Jaenisch, 1980; Jaenisch et al., 1981; Stewart et al., 1983; Soriano and Jaenisch, 1986). Mov mice release a continuous amount of infectious virus from a number of select tissues at predetermined times during ontogeny and early postnatal development. The Mov 14 strain carries the transgene on the X chromosome and activates the virus on embryonic day 14. Upon release, virus infects susceptible target cells exogenously. Within 3–6 months of age, Mov mice die of typical T-cell malignancies. Mov mice have been used to evaluate perinatal and transplacental AZT therapy. When transgenic Mov 14 pregnant females were treated with AZT throughout gestation and during lactation, offspring were protected from T-cell malignancies only when therapy was initiated during gestation. AZT therapy, however, was not effective when treatment was started after birth. Interestingly, AZT therapy during gestation was not associated with embryo toxicity or increased mortality (Sharpe et al., 1988).

Moloney murine sarcoma virus (MSV)

Moloney murine sarcoma virus (MSV) (Weiss et al., 1985) infection of mice has proven to be a very practical animal model to evaluate antiviral agents. MSV is a complex consisting of a replication-competent MoMuLV and a defective pathogenic virus which was generated by the insertion of the *mos* oncogene into the *pol* and *env* region of the helper virus. The MSV complex induces rhabdomyosarcomas in newborn and adult mice. Subcutaneous injection of newborn mice leads to tumor formation within 4–5 days, and the animals usually succumb 10–12 days post-inoculation. Tumor regression in adult mice is due to cellular immune responses mediated by $CD4^+$ T-helper cells. Moreover, virus spread and MSV-induced tumor growth (Balzarini et al., 1990; Balzarini et al., 1991) are associated with functional depletion of $CD4^+$ T-cells (Biasi et al., 1990; Bateman et al., 1987). Tumor size as well as host survival are related to the titer of the original viral inoculum and therefore provide convenient measurable parameters which are related to the efficacy of antiviral agents.

MuLV systems in drug development: caveats

In vivo drug assessment using MuLV systems has several advantages. Inbred host animals are available with well characterized genetics and immune systems so that host immune defense mechanisms can be studied during retroviral infections, and viral isolates can be quantitated. Furthermore, the latent period is relatively short, MuLV-infected mice pose no significant biohazard problems, require no special housing and are relatively inexpensive.

However, there are several concerns regarding drug development in MuLV systems. As Type C retroviruses, they lack lentiviral regulatory genes, require different cellular receptors and differ in pathogenicity from HIV-1. A candidate antiretroviral agent can only be considered for in vivo evaluation in MuLV systems when the following criteria are met:

(1) The candidate antiviral agent must inhibit a similar viral function(s) in both HIV-1 and MuLVs;

(2) Candidate antiretroviral agents must inhibit HIV-1 replication in vitro to approximately the same degree as that of MuLVs;

(3) Drug metabolism in human and mouse cells must be comparable;

(4) Pharmacokinetics in mice and humans should be similar;

(5) If an oncogenic murine retrovirus is used as a test virus, control

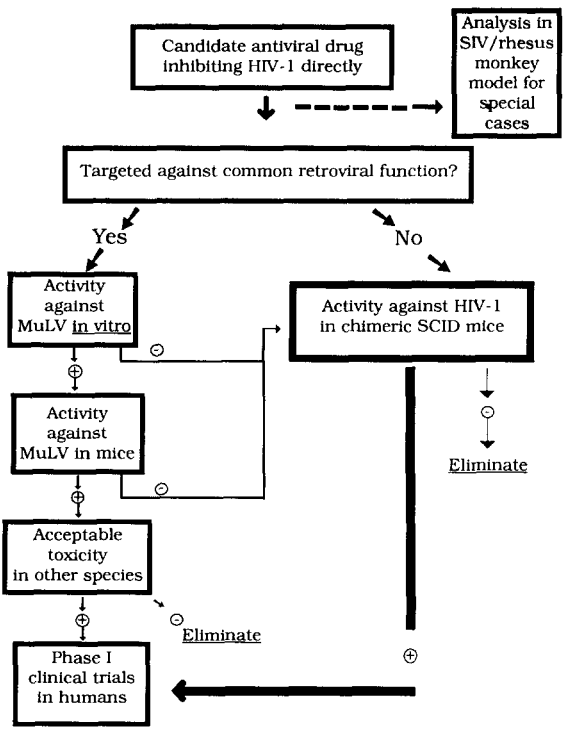

Fig. 1. Diagram summarizing the preclinical evaluation of a candidate anti-HIV-1 drug.

experiments must be conducted to rule out antineoplastic activity as the cause for a favorable clinical outcome as opposed to a purely antiviral activity of the inhibitor.

A diagram summarizing the preclinical evaluation of a candidate anti-HIV-1 drug is shown in Fig. 1. First, a candidate drug is screened in vitro against HIV-1 in various human cell lines and human peripheral blood mononuclear cells and must inhibit virus replication with relatively little toxicity. None of the animal models discussed should be used as a primary screening method. If a number of candidate antiviral agents are simultaneously being developed, animal models could be helpful in selection of the drug for clinical trials. Drugs that exhibit excessive toxicity at virus-inhibitory concentration could be eliminated. However, in order to select the appropriate animal model system for retroviral infection and/or disease, the following questions need to be addressed: Does the drug inhibit a common retroviral function or a lentiviral regulatory protein? Can the drug inhibit the animal virus equally well in vitro as HIV-1? If a common retroviral function is targeted by the inhibitor, MuLV models could be considered to evaluate the compound in vivo, and the agent could then be entered into phase I clinical trials if it is active and relatively non-toxic. Alternatively, if the compound is inactive against MuLVs either in vitro or in vivo, it should not be eliminated but rather considered for analysis in an alternate system. The same holds true if the inhibitor blocks a typical lentiviral function. In such circumstances, analysis in a SCID mouse model is most cost-effective. A drug found to be inactive and/or toxic in a SCID mouse system would be eliminated, but active and relatively non-toxic agents could be entered into phase I clinical trials.

Immunomodulatory agents, in contrast to antiretroviral drugs, may be inactive in vitro, since they interfere with HIV-1 replication through a cascade of cytokines that is fully functional only in an intact organism. Therefore, animal models of acquired immunodeficiency may have to be considered as a first step to evaluate this class of compounds. The most appropriate animal model to assess immunomodulatory agents appears to be SIV infection of rhesus monkeys due to the similarity between SIV and HIV-1 in terms of pathobiology.

The evaluation of prodrugs represents a special case. By definition, such compounds require metabolic conversion into an active form, which may or may not take place in cultured cells. As a consequence, definitive proof of anti-HIV-1 activity of a prodrug can only be obtained in an animal model system. Prodrugs represent a class of compounds that should not be eliminated after negative data are obtained in the tissue culture systems, and one could argue that prodrugs should undergo primary screening in an animal model system. Chimeric mouse systems seem well suited for such tasks.

To conclude, this summary gives an overview of animal retroviral model systems available currently for drug development against AIDS. The potential advantages and disadvantages of each system is outlined for the preclinical development of anti-AIDS therapy.

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