Murine Models for Evaluating Antiretroviral Therapy

Ruth M. Ruprecht, Lisa D. Bernard, Ting-Chao Chou, Miguel A. Gama Sosa, Fatemeh Fazely, John Koch, Prem L. Sharma, and Steve Mullaney

Division of Cancer Pharmacology, Dana-Farber Cancer Institute [R. M. R., L. D. B., M. A. G. S., F. F., J. K., P. L. S., S. M.]; Departments of Medicine [R. M. R.], Pathology [M. A. G. S.], and Biological Chemistry and Molecular Pharmacology [F. F., J. K., P. L. S.], Harvard Medical School, Boston, Massachusetts 02115, and Memorial Sloan-Kettering Cancer Center [T-C. C.], New York, New York 10021

Abstract

The pandemic of the acquired immunodeficiency syndrome (AIDS), caused by the human immunodeficiency virus type 1 (HIV-1), requires rapid development of effective therapy and prevention. Analysis of candidate anti-HIV-1 drugs in animals is problematic since no ideal animal model for HIV-1 infection and disease exists. For many reasons, including small size, availability of inbred strains, immunological reagents, and lymphokines, murine systems have been used for in vivo analysis of antiretroviral agents. Here we review currently available murine models involving HIV-1 in transgenic mice and in chimeric mice reconstituted with human cells, as well as murine systems using retroviruses of the subfamily Oncovirinae rather than Lentivirinae. We report our results on various antiretroviral treatment strategies, including chemoprophylaxis after acute retroviral exposure, therapy of chronic viremia, quantitative analysis of combination therapy, and therapy during pregnancy and in the neonatal period aimed at preventing viremia in the offspring. Due to our highly effective postexposure treatment protocols with 3'-azido-3'-deoxythymidine (zidovudine) combined with recombinant human interferon-α/β, retrovirus-inoculated mice developed immunity to the virus to which they were exposed, which will allow us to determine the nature of protective antiretroviral immunity in inbred mice.

Introduction

Major efforts have been undertaken by academic institutions as well as by the pharmaceutical industry to find effective therapy against HIV-1, the accepted cause of AIDS (1–3). While at the present time only AZT (zidovudine) has been approved by the Food and Drug Administration for treating AIDS and related conditions, several other drugs are being evaluated in clinical trials, and many more agents have proven anti-HIV-1 activity in vitro. A practical small animal model system for HIV-1 viremia and disease, in which potential anti-HIV-1 activity in vitro may be evaluated in the different model systems are outlined in Table 1.

Prior to using a given animal model system to test a candidate anti-AIDS agent, the following questions need to be addressed: (a) Is the potential anti-AIDS drug an antiviral agent or an immunomodulator that may be able to restore certain immune functions? Thus, is a candidate anti-AIDS drug likely to be active during the phase of asymptomatic viremia or only during overt disease? (b) Does a candidate antiviral agent inhibit a retroviral function shared by all retroviruses such as gag, pol, protease, integrase, RNase H, or env, or is the novel compound targeted against a regulatory gene product typical for lentiviruses (e.g., tat, rev, nef, or others)? (c) Does the metabolism of a test drug in animal cells resemble that in human cells? (d) Is the quantity of test drug available limited? Prior to in vivo testing, the biocontainment requirements for the planned animal experiments as well as projected costs need to be considered.

A well-defined experimental end point must be used for drug studies in animal model systems. Examples of such measurable parameters may include prevention of primary infection via postexposure prophylaxis, a decrease of virus load in chronically viremic animals, a delay in the development of overt disease, or prolonged overall survival.

Therapy aimed at preventing infection after acute virus exposure addresses a different clinical problem than therapy of chronic viremia or virus-induced disease. In the first case, cure may be possible if the therapeutic intervention is started promptly after virus exposure and if integration of the HIV-1 provirus in host chromosomal DNA, an irreversible event, can be blocked (Fig. 1). Such a therapeutic approach must involve drugs capable of inhibiting early steps in the viral replication cycle. However, other agents may also play a role in postexposure chemoprophylaxis. For instance, biological response modifiers capable of boosting host immune defense mechanisms may lead to selective killing of infected cells expressing virus-specific antigens on their cell surface and, thus, may act synergistically with antiviral drugs.

Therapy of chronically viremic individuals can only be suppressive, however, unless a treatment modality is discovered that can destroy all cells harboring latent virus. As long as such curative therapy is lacking, antiviral treatment in chronic viremia needs to be administered indefinitely. Long-term tolerance and drug safety thus become important topics. Combination therapy may eventually play a major role in the therapy of chronic viremia not only by improving the therapeutic window, but also by preventing selection of drug-resistant virus strains.

Using inbred laboratory mice for drug development would be advantageous because of well-studied genetics, immunology, hematopoiesis, and metabolism, and the availability of many immunological reagents. Furthermore, breeding can be accomplished easily because of the short generation time, and the costs for housing laboratory mice are considerably lower than for other experimental animals. Because of the small size of mice, drug studies can be carried out with relatively small amounts of investigational compounds that may be limited in supply, especially in natural product analysis.

1 Presented at the "XIVth Symposium of the International Association for Comparative Research on Leukemia and Related Diseases," October 8–12, 1989, Vail, CO.

2 Supported by NIH Contract N01-AI-72664, NIH Grants U01-AI-24845 and R01AI29797-01, and a Faculty Research Award from the American Cancer Society to R. M. R., and NIH Grant U01-AI-26056 to T-C. C.

3 To whom requests for reprints should be addressed, at Dana-Farber Cancer Institute, 44 Binney Street, Boston, MA 02115.

4 The abbreviations used are: HIV-1, human immunodeficiency virus type 1; RLV, Rauscher murine leukemia virus; MoLV, Moloney murine leukemia virus; HTLV-I, human T-lymphotropic virus type I.

5618s
Unfortunately, cultured murine cells and mice cannot be infected by HIV-1. Since it is known that infection by this virus critically depends on human CD4 cell surface receptors, murine cells have been transfected with DNA encoding the human CD4 receptor sequences. Even though the transformed murine cells synthesized human CD4 receptor molecules faithfully, they were nevertheless refractory to HIV-1 infection (5). Apparently, one or several post-receptor binding steps independent of CD4 interaction are required for successful viral entry. However, cultured murine cells are able to produce infectious HIV-1 progeny following transfection of a molecular HIV-1 clone (6), demonstrating that direct proviral DNA insertion techniques can overcome the early block of HIV-1 infection of murine cells.

**Mature Systems Involving HIV-1**

**Transgenic Mice**

Transgenic mouse systems involving HIV-1 are summarized in Table 2. Transgenic mice have been created carrying either intact (7) or partial (8-10) HIV-1 proviral genomes. Activity of the HIV-1 LTR in Transgenic Mice. Leonard et al. (8) generated four lines of transgenic mice containing the reporter gene CAT under the transcriptional control of the HIV-1 LTR. In all lines, a distinct pattern of tissue-specific CAT expression was observed; CAT activity was detected in the eye, heart, spleen, thymus, and tail. In lymphocytes as well as in circulating monocytes, CAT expression could be stimulated by cocultivation with mitogens or various cytokines, respectively. Among cells derived from the monocyte-macrophage lineage, Langerhans cells of the skin had the highest levels of CAT activity, indicating that HIV-1 LTR-directed CAT activity may increase proportionally to the degree of differentiation of these cells. These transgenic mice may prove to be useful for the analysis of tissue-specific transcription factors interacting with the HIV-1 LTR.

**Transgenic Mice Expressing HIV-1 tat.** Khillan et al. (9) have obtained a line of transgenic mice carrying the bacterial gene CAT under the transcriptional control of the HIV-1 LTR. These mice were mated with transgenic animals of the opposite sex carrying the HIV-1 tat gene fused to mouse α-crystallin A

<table>
<thead>
<tr>
<th>Model system for HIV-1 infection</th>
<th>Therapeutic modality to be tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute virus exposure</td>
<td>Prevention with postexposure chemotherapies</td>
</tr>
<tr>
<td>Maternal transmission</td>
<td>Transplacental prophylactic therapy of the fetus, suppression of maternal virus production</td>
</tr>
<tr>
<td>Chronic viremia</td>
<td>Chronic suppressive therapy</td>
</tr>
<tr>
<td>HIV-1-induced disease</td>
<td>Antiviral therapy, immune restoration</td>
</tr>
<tr>
<td>Acquired immunodeficiency</td>
<td>Antiviral therapy, antimicrobial therapy + prophylaxis</td>
</tr>
<tr>
<td>Opportunistic infection</td>
<td>Cytotoxic therapy</td>
</tr>
<tr>
<td>Kaposis's sarcoma</td>
<td>Antiviral therapy, ? other</td>
</tr>
<tr>
<td>Nervous system disease</td>
<td>Antiviral therapy and/or therapeutic modalities for idiopathic thrombocytopenic purpura</td>
</tr>
<tr>
<td>Central</td>
<td>Antiviral therapy, ? other</td>
</tr>
<tr>
<td>Peripheral</td>
<td>Antiviral therapy, ? other</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>Antiviral therapy, ? other</td>
</tr>
</tbody>
</table>

**Table 1 Animal/retrovirus models for testing therapy**

The retroviral life cycle can be divided into early steps, which comprise events starting with viral adsorption to cellular receptors and ending in proviral DNA integration (1), and late events, which begin with transcription of the proviral DNA and result in the release of mature virions (2). After binding to a specific cellular receptor, the viral glycoprotein envelope (3) fuses with the cell membrane, thus allowing the viral core to enter the cytoplasm. The viral core contains two copies of the retroviral RNA genome as well as the necessary enzymes for complementary DNA synthesis and integration. Reverse transcription starts at the rRNA binding site (cloverleaf), and the final product is a linear, double-stranded proviral DNA molecule with identical LTRs, whereas template RNA molecules are degraded by RNase H. LTR building blocks U3, R, and U5 are symbolized by J (4) and 5, respectively, while the retroviral genes gag, protease, polymerase, integrase, and env are represented by GAPPE. Proviral DNA is integrated into chromosomal DNA by viral integrase while still particle associated, whereas supercoiled, closed circular DNA forms represent reaction side products. Proviral integration is irreversible and may lead to latency or active viral production. The late phase of viral replication is initiated by RNA polymerase II which synthesizes full-length RNA which may remain unspliced or may get spliced and polyadenylated. The latter RNA molecules are translated, and the resulting gene products may undergo posttranslational modification, such as glycoprotein processing or proteolytic cleavage by protease (4). Full-length RNA molecules are packaged into virions which are released by budding. As an example, the major replication steps of murine leukemia viruses have been shown. Lentiviral propagation differs by the action of regulatory genes which influence virus replication at various stages.

**Fig. 1.** The retroviral life cycle. Retroviral propagation can be divided into early steps, which comprise events starting with viral adsorption to cellular receptors and ending in proviral DNA integration (1), and late events, which begin with transcription of the proviral DNA and result in the release of mature virions (2). After binding to a specific cellular receptor, the viral glycoprotein envelope (3) fuses with the cell membrane, thus allowing the viral core to enter the cytoplasm. The viral core contains two copies of the retroviral RNA genome as well as the necessary enzymes for complementary DNA synthesis and integration. Reverse transcription starts at the rRNA binding site (cloverleaf), and the final product is a linear, double-stranded proviral DNA molecule with identical LTRs, whereas template RNA molecules are degraded by RNase H. LTR building blocks U3, R, and U5 are symbolized by J (4) and 5, respectively, while the retroviral genes gag, protease, polymerase, integrase, and env are represented by GAPPE. Proviral DNA is integrated into chromosomal DNA by viral integrase while still particle associated, whereas supercoiled, closed circular DNA forms represent reaction side products. Proviral integration is irreversible and may lead to latency or active viral production. The late phase of viral replication is initiated by RNA polymerase II which synthesizes full-length RNA which may remain unspliced or may get spliced and polyadenylated. The latter RNA molecules are translated, and the resulting gene products may undergo posttranslational modification, such as glycoprotein processing or proteolytic cleavage by protease (4). Full-length RNA molecules are packaged into virions which are released by budding. As an example, the major replication steps of murine leukemia viruses have been shown. Lentiviral propagation differs by the action of regulatory genes which influence virus replication at various stages.
transcriptional control elements. In the F1 offspring, transactivation of the CAT gene was seen only in the eye, the tissue in which the α-crystallin A promoter is active preferentially. These data demonstrate that mice can synthesize functional tat protein.

Transgenic mice expressing the HIV-1 tat gene linked to the HIV-1 LTR, which were generated by Vogel et al. (10), developed skin lesions resembling Kaposi’s sarcoma. Surprisingly, only male transgenic mice developed these skin lesions which consisted of spindle cell proliferation and tumor development, even though female mice synthesized similar levels of tat mRNA.

Transgenic Mice Producing Full-Length HIV-1. Transgenic mice carrying a complete HIV-1 provirus were described by Leonard et al. (7). No founder animals had detectable levels of HIV-1 viremia or signs of disease despite the presence of integrated HIV-1 proviral sequences. After female founder mice were mated with normal syngeneic males, F1 litters of one founder female were affected severely as manifested by stunted growth; perivascular pulmonary lymphocytic infiltrates; epidermal hyperplasia involving tail, ears, nose, and feet; and death within 25 days postnatally. Splenomegaly, lymphadenopathy, and thymic involution were noted, but there was no selective loss of CD4+ T-cells, the characteristic finding in human AIDS. All affected F1 mice but none of the normal littermates carried the HIV-1 provirus. All attempts to isolate HIV-1 were successful and resulted in HIV-1 virions that were able to infect human cells but not murine fibroblasts, since the latter lack human CD4 receptors. Thus, affected F1 mice produced HIV-1, which was unable to replicate in the transgenic animals.

The relevance of these HIV-1 provirus-carrying transgenic mice to human HIV-1 infection and AIDS is unclear. While different types of skin lesions are known to occur in AIDS patients, such as seborrheic-like dermatitis, psoriasis, as well as ichthyosis (11–13), they differ from the lesions observed in the transgenic mouse. In contrast, the pulmonary interstitial lymphoid infiltrates resemble those seen in adult AIDS patients with nonspecific interstitial pneumonitis (14). It is possible that the lethal congenital disease in HIV-1 F1 mice is a direct consequence of the expression of HIV-1 gene products, especially since the HIV-1 LTR has demonstrated transcriptional activity (8) in some of the target tissues affected in the HIV-1 F1 mice. On the other hand, HIV-1 proviral sequences may have integrated at a critical locus within mouse chromosomal DNA, thus disrupting its proper function. Insertional mutations have been noted in transgenic animals by Jaenisch (15).

Potential Role of Transgenic Mice in the Evaluation of Anti-HIV-1 Drugs. Possibly, the in vivo analysis of candidate antiviral drugs targeted against specific HIV-1 gene products such as tat may be carried out in an appropriate transgenic mouse system. A transgenic mouse model involving full-length HIV-1 provirus may be useful for special studies of late steps in the viral replication cycle. Since no viral replication takes place, the early steps of the viral life cycle have been bypassed, and therefore, antiviral agents or therapeutic maneuvers aimed at steps preceding HIV-1 integration cannot be evaluated in this model. Thus, only antiviral agents or therapeutic maneuvers aimed at late steps in the viral replication cycle such as HIV-1 RNA or protein synthesis, posttranslational modification, viral assembly, and budding may be analyzed in this system. Of note is that, shortly after the publication of these findings, all transgenic mice containing a full-length HIV-1 genome perished in a laboratory accident (7).

Chimeric Mice

The limitations of transgenic mouse models, which do not support HIV-1 replication, were overcome by several groups that replaced the murine hematopoietic cells in mice with severe combined immunodeficiency with human stem cells, thus creating mouse/human chimeras. Homozygous scid/scid mice were used as recipients for human stem cells by two groups. scid (severe combined immunodeficiency) is an autosomal recessive mutation that arose spontaneously in C.B-17 mice (16). Mice homozygous for this mutation, i.e., scid/scid (so-called SCID) mice, lack functional T- and B-cells (16–18). Southern blot analysis of Abelson murine leukemia virus-transformed B-cell precursors or spontaneous thymomas in SCID mice revealed highly aberrant DNA rearrangements at the immunoglobulin or T-cell receptor loci (19). This defect is caused by a faulty VDJ recombinase mechanism shared by precursor cells of both B- and T-cell lineages (19, 20).

McCune et al. (21) used human fetal tissues derived from liver, thymus, and lymph nodes to reconstitute SCID mice. After engrafting, differentiation of mature human T-cells and B-cells was noted. Subsequently, the chimeric (so-called SCID-hu) mice were infected successfully with HIV-1 (22), which spread within the human lymphoid organs in the chimeras. A combination of immunohistochemistry and in situ hybridization revealed HIV-1 RNA transcripts in most infected cells, but only a small fraction of the cells contained both detectable viral RNA and proteins.

Using a different approach, Mosier (23) injected human PBL i.p. into SCID mice, which resulted in stable, long-term engraftment of a functional human immune system (23). Transplanted human PBL increased in numbers and survived for at least 6 mo; reconstituted mice secreted human immunoglobulins spontaneously, and a specific human antibody response could be elicited following immunization with tetanus toxoid. All major human cell populations present normally in PBL were found...
in the blood and lymphoid tissue of the chimeras. This method of reconstituting the immune system in the SCID mice may prove to be highly useful for studying HIV-1 infection and viremia. Such studies are in progress (Ref. 24; Footnote 4).

Immunodeficient mice of the genotype bg/nu/xid were used by Kamel-Reid and Dick (25) to generate chimeric human immunodeficient mice. In irradiated recipient mice, at least two major barriers prevent growth of transplanted human bone marrow cells, namely, NK cells as well as LAK cells. Even immunodeficient mice, such as SCID or nude mice, still contain high levels of NK and LAK activity (26, 27). In bg/nu/xid mice, the influence of NK and LAK cells is diminished greatly. The nu mutation signifies a loss of thymic function, the bg mutation severely reduces the number of NK cells, and the xid mutation results in reduced numbers of LAK cells. Using irradiated mice with these triple mutations, Kamel-Reid and Dick were able to engraft human bone marrow cells and, in the resulting chimeric human immunodeficient mice, human macrophage progenitor cells survived for prolonged periods of time. These chimeras may become highly useful tools for studying human macrophage infection by HIV-1 in vivo.

The chimeric mouse systems are highly promising since HIV-1 viremia and anti-HIV-1 therapy can be studied (28). Whether HIV-1 pathogenesis can be examined as well is unknown at the present time. Thus, it is not clear whether chimeric mouse systems can be utilized as models for HIV-1-induced disease. Possibly, these immune-reconstituted animals may become immunodeficient again after a period of HIV-1 viremia due to a selective destruction of CD4+ human lymphocytes. While the chimeric mouse system offers some unique advantages, several drawbacks need to be considered. (a) The life span of the human xenograft may be limited. (b) Because infection is sustained entirely within the xenograft, no CNS infection or pathology has been found. (c) Studies involving in utero infection are not feasible because the fetus lacks the human xenograft. (d) Since mice are known to harbor endogenous retroviral sequences of xenotropic host range that may become activated, the potential of creating novel retroviruses with altered host spectrum and pathobiology must be kept in mind, and appropriate biosafety containment facilities must be used to conduct HIV-1 infections of chimeric SCID mice. Thus far, no evidence has been found that genetic recombination or phenotypic mixing has occurred in the SCID mouse systems (29), even though HIV-1 with altered tropism has been found in a human T-cell leukemia cell line that had become infected by a xenotropic murine leukemia virus when passed through irradiated nude mice (30).

Oncornavirus Mouse Models

Murine Retrovirology

Thus far, no rodent lentivirus has been isolated. Murine retrovirus isolates have been classified as ecotropic (i.e., viruses capable of growing in cells of the species of origin), xenotropic (endogenous viruses of the species of origin unable to replicate well in cells of that species because of a block at the receptor level but able to grow in cells of distant species), and amphotropic (retroviruses able to replicate well in cells of the host species of origin as well as those of distant species). Ecotropic MoLVs can be titrated in the XC plaque assay, a test based on syncytium formation (31), in contrast to amphotropic viruses. Most ecotropic retroviruses replicate at low levels in cells of closely related rodents such as hamsters or rats, but their replication is blocked in cells of higher mammals because of a lack of appropriate cell surface receptors. Many animal species contain complete or partially defective endogenous viruses which exhibit a xenotropic host range upon activation. Thus far, no xenotropic murine virus has been pathogenic in any animal. This contrasts sharply with ecotropic viruses which may have full pathogenic potential (for review, see Ref. 32).

Murine Systems for Testing Antiviral Drugs

When we initiated our studies, no practical small animal models for HIV-1 infection and disease were available, and thus, we used surrogate retrovirus models involving MuLVs (4). MuLV-infected animals first pass through a variably long period of asymptomatic viremia before developing overt disease, as has been observed for HIV-1. Surrogate murine retrovirus model systems may be used as models for retroviral viremia, as opposed to models for HIV-1-induced disease. However, since MuLV models can only mimic some aspects of HIV-1 infection and pathogenesis in humans, the therapeutic modalities that may be tested using MuLV infection of mice are restricted, and several caveats need to be considered. As typical type C retroviruses whose propagation depends on the six common retroviral genes gag, protease, polymerase, RNase H, integrase, and env, MuLVs do not contain the novel lentiviral regulatory genes such as tat, rev, nef, vpu, vpr, and vif. Thus, data obtained in MuLV-exposed mice can only be considered as potentially relevant to human HIV-1 infections if the following conditions are met: (a) candidate antiviral agents must block similar viral functions in both HIV-1 and the murine test virus; (b) in cultured cells, test compounds must inhibit HIV-1 replication to approximately the same degree as that of the MuLV; (c) drug metabolism in human and mouse cells must be comparable; (d) pharmacokinetics in mouse and humans should be similar; and (e) control experiments should be conducted to rule out an antineoplastic drug action rather than an antiretroviral mechanism. In MuLV-infected mice, therapy could lead to clinically significant benefits if a novel drug is targeted against MuLV-transformed cells and is cytotoxic rather than antiviral.

MuLV systems should not be used for primary screens. Thus, we not only compared the relative inhibition of murine retroviruses and HIV-1 in vitro prior to initiating animal experiments, but we also restricted the latter to candidate antiviral agents with confirmed efficacy against HIV-1 in cultured cells. Our in vivo experiments focused on various aspects of prevention and therapy of viremia. In various MuLV model systems, we studied the two main questions that follow:

1. Is Retroviral Viremia Preventable after Acute Virus Exposure by Prophylactic Drug Therapy? Is there a critical time window between virus exposure and onset of therapy? What is the role of combination therapy in postexposure chemoprophylaxis? Can antiviral therapy prevent maternal transmission of pathogenic retroviruses? Is such therapy teratogenic?
2. Can Antiviral Drug Therapy Favorably Influence the Clinical Course of a Chronically Viremic Animal? Can the onset of overt disease be delayed? What are the toxic effects of chronic suppressive therapy? Does the treated animal ultimately benefit from the therapeutic intervention by prolonged survival? What is the role of combination antiretroviral chemotherapy, and can it be analyzed quantitatively? Can the CNS sanctuary be treated effectively with antiviral agents?

To answer these questions, we have used RLV, MoMuLV, and the neurotropic retrovirus Cas-Br-E (for review, please see

* D. Mosier, personal communication.

5621s

Downloaded from cancerres.aacjournals.org on April 12, 2017. © 1990 American Association for Cancer Research.
Ref. 32) in susceptible strains of mice. The efficacy of chemotherapy after acute retrovirus exposure was first demonstrated in mice inoculated with RLV (33). A subsequent study in retrovirus-exposed cats confirmed these findings (34). In several studies, therapy of chronically viremic mice has been evaluated (33, 35, 36). Transplacental or neonatal therapy was analyzed using T-cell or neurotropic viruses (37–39). A brief description of the biology of these viruses as well as the rationale for using these agents follows.

**Rauscher Murine Leukemia Virus**

RLV (40) was selected as a model for retroviral viremia because of its quantitative aspects. RLV is a type C retrovirus complex consisting of at least two components, namely, a replication-competent helper virus which by itself induces B-cell lymphomas late in the life of infected animals, and a replication-defective Rauscher spleen focus-forming virus which causes a rapidly fatal erythroid disease similar to that induced by the Friend virus anemia strain. Initial rapid erythroid expansion is followed by the development of frank erythroleukemia. A major advantage of RLV is the susceptibility of adult animals to viremia and disease. Eight days after virus inoculation, in a number proportional to the virus titer, colonies are formed in the spleen. Each colony represents a successful viral hit; the RLV-induced disease is thus polyclonal. With continued viremia, splenomegaly develops which becomes palpable at 2 wk postinoculation. One wk later, the spleens are enlarged massively, and the animals succumb to erythroleukemia within 4 to 6 wk after inoculation. The degree of splenomegaly measured on Day 20 postinoculation is also proportional to the viral titer (41). Thus, splenomegaly represents an easy, quantitative parameter to assess viremia in RLV-exposed animals.

**Moloney Murine Leukemia Virus**

In contrast to RLV, MoMuLV is T-cell tropic and only causes disease in mice inoculated as neonates. With an incubation time of 3 to 6 mo, MoMuLV has a more protracted course than does RLV. We have performed a series of studies with transgenic mice (Mov mice) carrying MoMuLV proviral sequences which are activated at different time points during fetal or early postnatal development, depending on the transgenic strain (42–46). Such animals are programmed genetically to release a given amount of infectious MoMuLV. Viremia ensues, and new target cells are infected exogenously. All animals succumb to typical MoMuLV-induced leukemia/lymphoma at the age of 3 to 6 mo (Fig. 2). By studying strains in which MoMuLV sequences are activated during gestation, we have tested the feasibility of transplacental antiviral therapy (40). In another series of experiments, we have studied whether or not the natural passage of infectious MoMuLV from a viremic female to her offspring, which occurs almost exclusively via milk, can be prevented by antiviral therapy (39).

**The Neurotropic Virus Cas-Br-E**

The neurotropic virus Cas-Br-E was first isolated by Gardner et al. (47, 48) from the brain of a paralyzed wild mouse trapped in the Lake Casitas area in California. A sequestered population of wild mice (Mus musculus domesticus) in this region of California has a high prevalence of spontaneous lymphoma and hind limb paralysis. Cas-Br-E is an ecotropic, N-tropic virus that, like RLV and MoMuLV, can be titrated in the XC plaque assay (49, 50). Cas-Br-E induces 100% hind limb paralysis in susceptible strains of laboratory mice. The virus needs to be injected into newborn mice, since inoculation of adult animals does not lead to paralysis. Cas-Br-E has been molecularly cloned by Jolicoeur et al. (51). By mutational analysis, the potential to induce hind limb paralysis was mapped to sequences within the envelope glycoprotein gp70 (52, 53). Like the human retroviruses with known neurotropism, HIV-1 or HTLV-I, Cas-Br-E is passed via semen and milk. The target cell of this virus is thought to be a lymphocyte (Cas-Br-E induces B-cell or null-cell lymphomas in mouse strains that develop neoplasms instead of paralysis). Initially, the virus replicates in the spleen, and after a period of viremia, the infection spreads to the CNS.

In paralyzed animals, neuropathological analysis shows spongiform polioencephalomyelopathy in the anterior horns of the spinal cord, the dentate nucleus of the cerebellum, and the brain stem (54–57). The white matter is affected to a somewhat lesser degree. Characteristically, no inflammatory reaction is seen. Cas-Br-E-induced lesions show a remarkable resemblance to certain HIV-1-induced myelopathies. However, in the latter, the distribution of spongiform changes in the spinal cord differs because of a greater involvement of white matter (58). We have used Cas-Br-E infection to study antiviral therapy targeted to the CNS as well as during gestation and in the neonatal period (37).

**The Immunosuppressive Murine Leukemia Virus LP-BM5**

Since our experiments were initiated, the LP-BM5 murine retrovirus isolate causing immunodeficiency has been described (59), which consists of a mixture of viruses, namely, a replication-competent, ecotropic virus which is nonpathogenic, and a replication-defective virus which scores positive in the mink focus-forming assay. The immunodeficiency is associated with the presence of the latter. LP-BM5 causes polyclonal B-cell proliferation, hypergamma globulinemia, and loss of T-cell function. Infected animals develop lymphadenopathy and splenomegaly, and they die within 26 wk of opportunistic infections, lymphadenopathy, or other causes (60). Late in the course of disease, the B-cell proliferation is oligoclonal or results in malignant lymphoma (61). As a member of the oncornavirus

---

**Fig. 2. Viremia and disease in transgenic Mov mice.**

- **Provirin activation in a few cells** → **Release of infectious MoMuLV** → **Viremia** → **Exogenous infection of target cells** → **Malignant transformation of T cells**

- **By blocking secondary virus spread, AZT suppresses viremia and exogenous infection of target cells, thus preventing malignant transformation.**

- **Development of T-cell leukemia or lymphoma** → **Death**
rather than the lentivirus subfamily. LP-BM5 like other MuLVs discussed earlier lacks the special lentivirus regulatory genes. Unlike HIV-1, LP-BM5 does not replicate in T-cells. Thus, the pathogenesis of LP-BM5-induced immunodeficiency may differ significantly from HIV-1-induced AIDS. Until the exact mechanisms of HIV-1- and LP-BM5-induced immunodeficiency are understood, it is uncertain whether LP-BM5 infection of mice can be used as a model for HIV-1-induced disease, and the same caveats about using LP-BM5 as a model system for antiviral therapy should be considered that were discussed for other MuLVs previously.

Materials and Methods

Type C Murine Retroviruses. The neurotropic retrovirus Cas-Br-E and the T-cell tropic MoMuLV were propagated as described (37, 39). The erythropoietic and lymphotropic RLV (strain RVB3, originally obtained from Dr. Mette Strand, The Johns Hopkins University) was only passaged in vivo to avoid loss of pathogenicity. The virus was prepared from cell-free supernatants of spleen single cell suspensions as published (35). Virus stocks were "quick frozen" in liquid nitrogen. Virus titrations were carried out by using the XC assay (33).

Antiviral Agents. AZT was a gift of Dr. Sandra Nusinoff-Lehrman (Burroughs Wellcome Co.), and recombinant human interferon-α/D (which is highly active across species barriers and prevents RLV-induced viremia in mice) was a gift of Dr. Iain Sim (Hoffman-La Roche, Inc.).

Animal Experiments. The in vivo analysis of antiviral agents was carried out in the various systems as described (33, 35–39, 62). All test drugs were analyzed first in tissue culture systems for their ability to inhibit the appropriate murine viruses prior to initiating any animal experiments (4).

Immunoblot Analysis. To test blood samples or spleen extracts for the presence of RLV-specific antigens, we performed immunoblot analyses as published (62), using a goat anti-RLV antiserum (NIH, Bethesda, MD).

Results and Discussion

Prevention of Viremia and Disease with Chemoprophylaxis after Retroviral Exposure

After accidental needle stick exposure to body fluids from AIDS patients, the HIV-1 seroconversion rate is approximately 1 in 200. No information will be available regarding the size of the inoculum, the depth of penetration from the sharp object, or particular virus strain (63). In areas with a high incidence of AIDS, health care workers are under added stress because of the fear of contagion. The level of anxiety could be lowered if a treatment program with demonstrated efficacy were available after accidental exposure to contaminated material.

We have tested the feasibility of postexposure chemoprophylaxis in mice inoculated i.v. with RLV. If therapy with high-dose AZT (150 mg/kg/day, given p.o. in the drinking water) was initiated 4 h postinoculation and continued for 3 wk, the development of splenomegaly was inhibited effectively (33). We could not find infectious virus in the plasma of virus-exposed, AZT-treated animals after the course of therapy was completed. Likewise, we were unable to detect infectious splenocytes in the XC infectious center assay. However, this AZT regimen caused anemia by the end of therapy.

Since AZT is targeted against reverse transcriptase, an enzyme with a critical function in the early part of the retroviral life cycle, we tested how soon after virus exposure therapy would have to begin to result in effective suppression of viremia and disease. Groups of mice were inoculated i.v. with RLV, and AZT therapy was initiated after various time intervals. On Day 21 postinoculation, the inhibition of splenomegaly as compared with RLV-inoculated, untreated animals was determined (Table 3). The onset of AZT therapy within 4 days of RLV exposure was still able to suppress disease, in contrast to later starting times. AZT therapy of mice already exhibiting massive splenomegaly did not result in a significant decrease of spleen weights, indicating that AZT was not acting via an antineoplastic mechanism in the RLV system (33).

We also tested a lower dose of AZT, which by itself inhibited RLV-induced splenomegaly by 90% but was not associated with the development of anemia. One cohort of animals was exposed to RLV at Time 0 and given this low dose of AZT starting 4 h postinoculation continuously for their life span. All animals tolerated the AZT well, and all were long-term survivors 2.5 yr after the start of the experiment. Intermittent virological examinations failed to reveal any infectious virus in the plasma of the AZT-treated animals, and at necropsy, no lesions characteristic of RLV were seen.

In another set of experiments (see below), we had identified the combination of AZT and recombinant human interferon-α/D as highly synergistic in RLV-exposed mice (62). We have tested this combination for its ability to prevent RLV infection after acute virus exposure (62, 64). A 20-day course of therapy with this regimen showed the following: (a) The development of splenomegaly was inhibited effectively in all virus-exposed, treated mice. (b) No infectious RLV could be found in the plasma or splenocytes of these animals after completion of therapy (c) No RLV-specific RNA sequences and no RLV-related antigens were detected in the spleens of virus-exposed animals after the treatment course (Fig. 3).

We have demonstrated the effectiveness of postexposure chemoprophylaxis in RLV-exposed animals. These results are highly significant for developing treatment protocols for HIV-1-exposed humans. Our findings have led to the design of clinical trials of postexposure AZT for laboratory or health care workers accidentally exposed to HIV-1 (65).

Antiretroviral Combination Therapy in Murine Models

Combination therapy may provide the most promising approach to long-term control of retroviral viremia. Agents with different modes of inhibiting retroviral propagation and nonoverlapping host toxicity could be used in combination regimens, following the principles used with great success in clinical oncology. Because of the emergence of AZT-resistant strains of HIV-1 (66, 67), the development and evaluation of combination therapy have become urgent clinical problems.

We have used RLV infection of mice to analyze the drug interactions between the reverse transcriptase inhibitor AZT and the protease inhibitor saquinavir (SQV) in murine retroviral models. RLV infection is used as a model for HIV-1-induced disease, and the same caveats about using LP-BM5 as a model system for antiviral therapy should be considered that were discussed for other MuLVs previously.

Table 3. Start of AZT therapy after RLV exposure

<table>
<thead>
<tr>
<th>AZT Groups</th>
<th>Virus (1 mg/ml)</th>
<th>Start</th>
<th>% of inhibition of splenomegaly</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>+</td>
<td>–</td>
<td>96.9</td>
<td>&lt;0.0001 (A vs. C)</td>
</tr>
<tr>
<td>B</td>
<td>–</td>
<td>–</td>
<td>98.6</td>
<td>&lt;0.0001 (A vs. D)</td>
</tr>
<tr>
<td>C</td>
<td>+</td>
<td>4 h</td>
<td>74.1</td>
<td>0.001 (C vs. E) Joules</td>
</tr>
<tr>
<td>D</td>
<td>+</td>
<td>4 days</td>
<td>9.9</td>
<td>Not significant (A vs. F)</td>
</tr>
<tr>
<td>E</td>
<td>+</td>
<td>8 days</td>
<td>9.9</td>
<td>Not significant (A vs. F)</td>
</tr>
<tr>
<td>F</td>
<td>+</td>
<td>19 days</td>
<td>74.1</td>
<td>0.001 (C vs. E) Joules</td>
</tr>
</tbody>
</table>

We have tested this combination for its ability to prevent RLV infection after acute virus exposure (62, 64). A 20-day course of therapy with this regimen showed the following: (a) The development of splenomegaly was inhibited effectively in all virus-exposed, treated mice. (b) No infectious RLV could be found in the plasma or splenocytes of these animals after completion of therapy (c) No RLV-specific RNA sequences and no RLV-related antigens were detected in the spleens of virus-exposed animals after the treatment course (Fig. 3).

We have demonstrated the effectiveness of postexposure chemoprophylaxis in RLV-exposed animals. These results are highly significant for developing treatment protocols for HIV-1-exposed humans. Our findings have led to the design of clinical trials of postexposure AZT for laboratory or health care workers accidentally exposed to HIV-1 (65).
and recombinant human interferon-αA/D, a biological response modifier with several antiviral and immunomodulatory activities. Both AZT and recombinant human interferon-αA/D inhibited RLV-induced splenomegaly in a dose-dependent fashion in animals exposed de novo to RLV (62). When used in combination, AZT and recombinant human interferon-αA/D were highly synergistic, as analyzed by the median effect plot and combination index method of Chou and Talalay (68).

We were able to lower the dose of both agents 10- to 60-fold in the combination regimens as compared with single-agent therapy, while maintaining the same biological end point, i.e., inhibition of splenomegaly. No significant bone marrow toxicity was observed in a 3-wk course of therapy which resulted in greater than 93% inhibition of virus-induced splenomegaly (62).

We have demonstrated that drug interactions in combination regimens can be calculated in retrovirus-exposed animals by using the quantitative RLV system.

**Acquired Immunity to Pathogenic Retrovirus**

RLV-exposed mice could be protected from viremia and disease with a combination regimen of AZT and recombinant human interferon-αA/D administered for 20 days. To test how the RLV-exposed mice processed the antigenic load given with the RLV inoculum, we performed the following pilot experiment (Fig. 4). Groups of mice were started on AZT and recombinant human interferon-αA/D at −3 days, and at Time 0, they were inoculated with either saline or live RLV. Antiviral therapy was continued until Day 20 postinoculation. After cessation of therapy, all animals were again given injections of live RLV but left without further antiviral drug therapy. All mice that had received saline at Time 0 developed splenomegaly and died. In contrast, all mice given live RLV at Time 0 remained healthy. On Day 60, these animals received another RLV rechallenge, and as controls, a group of naive mice was given the same virus inoculum. All of these control animals developed splenomegaly, whereas five of six mice initially exposed to RLV (Group 5b) resisted RLV rechallenge. In effect, we have vaccinated these experimental animals with a live, pathogenic retrovirus that was inhibited in its propagation by a highly effective antiviral combination regimen (62). Because this discovery was made in inbred mice, we plan to use passive immunization to determine the nature of the protective retroviral immune response.

**Neurotropic Retroviruses: Pathogenesis and Therapeutic Approaches**

Presently, two retroviruses are accepted as the etiological agents of human disease, namely, HIV-1 and the HTLV-I (69).
Both agents cause CNS damage; the latter virus has been implicated as the causative agent of tropical spastic paraparesis, a chronic progressive myelopathy (70-72). Any therapeutic strategy aimed at inhibiting the propagation of these agents must be able to reach the CNS sanctuary.

We have developed a murine model that allows us to study the pathobiology and therapeutic approaches to retrovirus-induced CNS disease (37). This model involves injection of the neurotropic murine leukemia virus Cas-Br-E during midgestation or in the neonatal period into susceptible SWR/J mice, all of which develop hind limb paralysis.

To test whether infection of developing embryos at the time of neural tube formation alters the pattern of paralysis, midgestation embryos were inoculated with Cas-Br-E virus-producing cells (37). This resulted in a marked acceleration of the disease process; infected animals developed tremor 2 wk postnatally and died at 3 to 4 wk of age. In contrast, neonatal infection resulted in tremor and paralysis at 4 to 6 mo of age. Interestingly, virus exposure during the time of neural tube formation resulted in the same extent of disease; paralysis was still restricted to the hind limbs.

Cas-Br-E infection of midgestation embryos and neonatal mice was used to study the impact of AZT therapy on onset and progression of CNS disease (37). In animals infected as newborns, continuous AZT therapy resulted in longer disease-free survival as compared with untreated, infected animals or animals receiving only a 3-wk course of AZT. In contrast, when AZT therapy of animals inoculated with Cas-Br-E as neonates was delayed for 2 mo and started only shortly before the onset of disease, we could no longer detect any therapeutic benefit.3 This implies that reinfection of new CNS target cells may not represent the main mechanism of Cas-Br-E neurovirulence, and that a reservoir of infected cells actively synthesizing and releasing virions or viral gene products into the CNS sanctuary may be responsible for the CNS damage. Since AZT is active only at an early viral replication step, it would be expected neither to decrease transcription of integrated proviral sequences nor to have any influence on disease progression after a critical number of cells has been infected.

Antiviral agents targeted at late steps in the viral life cycle and, thus, capable of inhibiting virus production may be used as probes to elucidate the mechanism of CNS damage in this model. The Cas-Br-E model may be applied also to evaluate candidate antiviral agents for activity in the CNS sanctuary. Specifically, lipophilic prodrugs designed to penetrate the blood/brain barrier easily and to be converted into active antiviral agents in the CNS sanctuary need to be evaluated in vivo, since no other assay system is available to measure prodrug activity in vitro. To summarize, our model represents a valuable tool for the study of retroviral neurovirulence as well as for testing approaches to treat retroviral CNS infection.

Therapy and Prevention of Perinatal Transmission of Pathogenic Retroviruses

In January 1988, 1 of 61 babies born in the New York City area had antibodies to HIV-1 (73), and since then, certain boroughs in New York City are reporting a rate of 10% of seropositive newborns. Since 35% to 50% of such infants are truly infected, a significant number of pediatric AIDS cases is expected. Infection may occur transplacentally, during delivery, or via mother’s milk. To date, nothing can be done to prevent or treat perinatally acquired HIV-1 infection other than avoiding nursing of the infant.

We have initiated studies aimed at preventing maternal transmission of pathogenic retroviruses (37-39); we have developed murine models for analyzing antiviral therapy across the placenta and in the neonatal period. Because no murine lentivirus is known, we performed our studies of transplacental antiviral chemotherapy with type C retroviruses, namely, the neurotropic virus Cas-Br-E and the T-cell tropic Moloney murine leukemia virus.

We have studied in two murine models whether antiviral therapy can be used to treat perinatal infection with pathogenic retroviruses. Our results demonstrate (37, 74) the following.

(a) AZT effectively crosses the placenta. (b) In mice infected as midgestation embryos with Cas-Br-E, AZT therapy led to a dose-dependent delay of neurological disease as well as a highly significant prolongation of life. As a result of AZT therapy, the disease in viremic animals progressed at a much slower pace. (c) No teratogenicity or developmental toxicity was seen in animals exposed prenatally as well as during early postnatal development to continuous AZT therapy.

In another set of experiments, we have examined maternal transmission of infectious MoMuLV from chronically viremic females to their offspring (39). We found that very few, if any, viruses pass before or during birth, and that most of the infection is acquired via virus-containing milk. We used this model to test the effect of AZT on natural maternal transmission of MoMuLV. As expected, AZT treatment of viremic mothers did not affect virus titers in the milk, but it suppressed viremia in the majority of pups. In 75% of the offspring, no evidence of virus infection was found by radioimmunossay to the gag protein p30. In the remaining 25%, AZT therapy did not prevent maternal transmission of MoMuLV but nevertheless markedly reduced the virus load in the infected offspring.

To study retroviral infection during pregnancy, we have used Mov animals carrying the MoMuLV provirus in their germ lines (38). Expression of the endogenous MoMuLV genome begins in a few cells and results in the release of infectious virions (Fig. 2). From the initial site of activation, the infection spreads and leads to the development of viremia and eventually to T-cell leukemia/lymphoma. Prenatal virus activation mimics transplacental infection, since virus gene expression and spread begin in utero.

The aim of our study was to develop a rapid, convenient model system for evaluating therapy aimed at the prevention of retroviral infection acquired prenatally, intrapartum, or in the neonatal period. We have used AZT as the test agent in these various Mov strains, and we have tested its effects on the development of viremia and subsequent T-cell leukemia/lymphoma. In parallel, all animals were monitored for evidence of embryo toxicity or developmental abnormalities. In six of seven Mov strains tested, AZT therapy led to marked improvement in survival and delayed the onset of T-cell leukemia/lymphoma significantly. AZT therapy led to the most significant therapeutic benefits in transgenic strains that activate MoMuLV after birth, whereas in those with virus activation prior to birth, AZT effects were variable. Among the latter strains, Mov 14 animals derived a highly significant therapeutic benefit from transplacental AZT therapy. We have shown that Mov 14 mice can be used to study the biological effectiveness of transplacental antiviral therapy.

In summary, we have tested the possibility of preventing and/or treating perinatal retroviral infections. The results of our preclinical studies are highly significant for planning treatment...

---

3 Unpublished observation.
strategies aimed at preventing maternal transmission in retrovirus-infected women. We have established several practical, cost-effective small animal models that allow testing various treatment strategies quickly and effectively. Our preclinical results have been used to plan a clinical trial in asymptomatic HIV-1-positive pregnant women who will receive AZT from the 28th wk of gestation and during delivery. This Phase I study is presently ongoing.6

References


* Y. Bryson, University of California, Los Angeles, personal communication.
Murine Models for Evaluating Antiretroviral Therapy
Ruth M. Ruprecht, Lisa D. Bernard, Ting-Chao Chou, et al.

Cancer Res 1990;50:5618s-5627s.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/50/17_Supplement/5618s

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.