AIDS RESEARCH

Animal Models of HIV-1 Disease

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TECH VIEW

Animal models provide a controlled setting for the study of human immunodeficiency virus–1 (HIV-1) disease, the preclinical testing of novel antiviral compounds, and the evaluation of vaccines (Fig. 1). Because the animals serve as models for humans, they should be closely reflective of human physiology and pathophysiology (*1*). Moreover, their use must be complementary to, and not replaceable by, experimental approaches that do not require animals. For any given experiment, the critical question is: which, if any, model is most useful—and why?

The history of the SCID-hu mouse provides an example of animal model development and assessment of utility. This model was initially imagined to be applicable across a broad investigative swath (*2*). After implantation of immunodeficient C.B-17 *scid/scid* (SCID or severe combined immunodeficiency disease) mice with human fetal liver, thymus, and lymph node tissue (Fig. 2), it was postulated that the implants would grow and become tolerant of the mouse environment. Reciprocally, it was believed that the immunocompromised status of the mouse recipient would permit such growth. If so, multilineage human stem cells derived from the fetal liver would differentiate, and mature single-positive T cell progeny might then migrate into implants of human peripheral lymphoid tissue, creating a chimeric mouse with a human immune system.

The outcome of this experiment was more straightforward than initially anticipated: SCID-hu (also called Thy/Liv) mice transplanted with human fetal liver and fetal thymus demonstrated long-term multilineage human hematopoiesis, including T lymphopoiesis, and the engrafted animals appeared to be functionally less immunodeficient than nonengrafted littermates. Given these preliminary data, a decade of intensive evaluation circumscribed those areas in which the SCID-hu mouse has actual utility:

1) *The analysis of normal human hematopoiesis*. Human thymus implants were initially shown to support the multilineage differentiation of early hematopoietic progenitor cells and, together with a human bone implant model, were pivotal in the identification of a candidate human hematopoietic stem cell (*3*). Additionally, these models have been used to analyze the effects of exogenously provided, species-specific human cytokines and for the definition of events associated with human thymopoiesis (*4*).

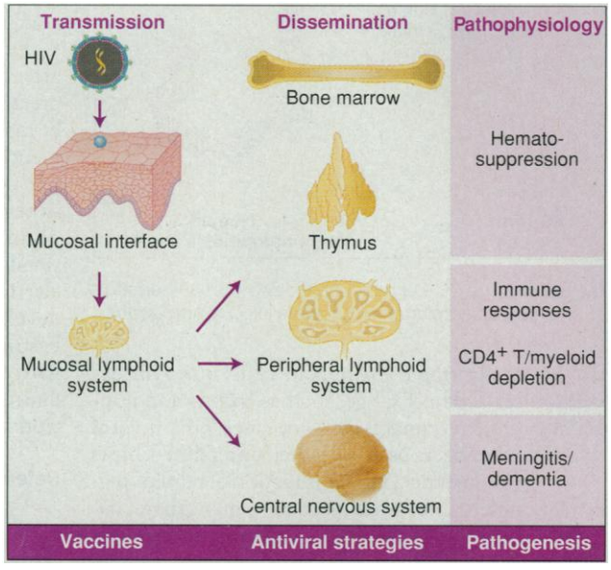

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Fig. 1. Modeling HIV-1 disease. The schematic shows a series of events that might occur after infection across a mucosal interface, with dissemination of HIV-1 (either as free or cell-associated virions, or both) to peripheral lymphoid organs, central hematolymphoid organs (such as bone marrow and thymus), and the central nervous system.

2) *The evaluation of HIV-1 infection in the human thymus*. The Thy/Liv model provides one of the only settings to evaluate HIV-1 effects on human thymopoiesis in vivo. Upon challenge with HIV-1, it was observed that tissue culture–adapted isolates of HIV-1 were not infectious in the model, whereas primary isolates were uniformly infectious. Derivative studies have demonstrated that certain subgenomic regions of HIV-1 (nef, for example) are critical for replication in vivo (*5*, *6*). It has also been noted that some isolates are infectious but not pathogenic in the Thy/Liv organ, prompting evaluation of differential HIV-1 pathology, which might be related to differences in cell tropism (*7*, *8*). Studies of this type, not possible in humans, contribute information important to the analysis of T cell turnover in HIV-1 disease.

3) *The evaluation of antiviral compounds*. Short-term challenge assays for both HIV-1 and cytomegalovirus have been devised in the SCID-hu mouse (*9*, *10*). These assays involve cohorts of 40 to 50 mice made with fetal tissue from a single donor and permit comparison among six to seven dosing groups of five to eight mice each. Given such group sizes and appropriate controls, it is possible to compare the activities of related congeners with statistical precision. Such information can contribute to the decision-making process in the preclinical drug development pathway.

4) *The development of human hematopoietic stem cell (HSC)–based gene therapy approaches against HIV-1*. Introduction of exogenous "anti–HIV-1 genes" into hematopoietic stem cells represents a possible long-term strategy for the treatment of HIV-1 disease. The SCID-hu models uniquely encompass the preclinical tests required for this approach (*11*). Such tests are serving to move this potential therapy toward the clinic (*12*, *13*).

Under the best of circumstances, data from the SCID-hu mouse model must be interpreted with several caveats in mind. The physiology of the human Thy/Liv and bone implants is likely perturbed by the surrounding mouse environment: mouse cells migrate into the grafts, and mouse-derived factors (such as cytokines, vitamins, and trace elements), which permeate the human implants, may not be appropriate or sufficient for optimal function of the cells therein. Conversely, necessary human factors may be lacking. SCID mouse colonies are vulnerable to opportunistic infections. Finally, it is fetal human implants that are functional in this model, and data sets may not be directly transferrable to adult physiology.

There are also experimental arenas in which the SCID-hu mouse is definitely not useful. Most important among these is evaluation of the peripheral human immune system. Human lymph nodes implanted into many locations of the mouse become grossly disorganized within a short period of time, likely the result of graft-versus-host reactions, host-versus-graft reactions, or the lack of appropriate lymphatic and vascular connections, or a combination of these. These structures are not models of normal human physiology.

Other strategies have been advanced for the production of chimeric mouse models for HIV-1 disease, most of which have not re-

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ceived the same degree of scrutiny as the SCID-hu mouse. In those which use transfers of adult human peripheral blood mononuclear cells (*14*), however, graft-versus-host reactions prevail, and persisting human cells become anergic in vivo (*15*). Chimeric mouse models using this approach are thus suboptimal for the evaluation of de novo human immune responses.

These problems focus attention on other animal models of HIV-1 disease (*16–19*), particularly for the evaluation of vaccines and pathogenic mechanisms. Alternative models include animals that are studied after natural or experimental infection with animal lentiviruses, after inoculation of HIV-1 or HIV-2 or recombinants carrying subgenomic regions thereof, or in the context of HIV-1 transgenes. Each of these approaches carries advantages and disadvantages:

1) *Transgenic mouse models*. Transgenic mouse stocks have been prepared for the analysis of specific aspects of HIV-1 replication. In these models, the interaction of individual viral proteins with host factors can be studied in selected cell types in vivo. They would appear to be useful for testing certain antiviral compounds, especially with respect to bioavailability and mechanism of action in vivo, but this application has not been successfully pursued to date. Given the many dissimilarities with the human immune system, it is unlikely that these models will play a major role in vaccine development.

2) *Animal lentivirus models*. Lentiviruses were first studied as natural infections of horses and sheep. Other animal lentiviruses have been examined in goats, cattle, cats, and nonhuman primates. These animals, and especially those infected with the diverse group of simian immunodeficiency viruses (SIV), have proven useful in the analysis of the pathogenesis and transmission of their respective lentiviral agents. SIV infection of rhesus macaques recapitulates many of the pathologic features of HIV-1 disease in humans. Such models cannot, however, serve as complete mimics of HIV-1–induced pathology in humans. The genomic organization of animal lentiviruses is similar to but not entirely homologous with that of HIV-1, and important features of pathogenesis are not shared by all. Drugs and vaccines designed to inhibit HIV-1 replication may not be effective against related but nonidentical viral targets. Even if they were, these animals are not easily arranged into cohort sizes large enough to test candidate antiviral compounds in a statistically significant manner.

3) *HIV infection models*. These models superimpose HIV-1 or HIV-2 (viruses normally tropic for human cells) onto an extended host range, such as rabbits and nonhuman primates. In some cases, HIV isolates have been used to infect unmodified hosts (for instance, HIV-1 infection of rabbits, pigtailed and rhesus macaques, mangabeys, baboons, or chimpanzees). In others, the host is genetically modified to express the human receptor for HIV (for instance, CD4-transgenic rabbits) or the challenge HIV isolate is genetically altered such that it is better able to replicate in the nonhuman host (for example, "SHIV" recombinants between SIV and HIV-1 introduced into rhesus macaques or baboons). These models are of interest because they permit analysis of infections with HIV or subgenomic regions thereof. This facilitates direct preclinical analysis of candidate antiviral compounds or vaccines and may also reveal aspects of viral pathogenesis not observed with other animal lentiviruses. These models are nonetheless marked by several problems. First, in the same manner that HIV isolates are attenuated upon passage in tissue culture, adaptation in a heterologous host may lead to the selection of variants with unknown and potentially irrelevant properties. Second, although these models can be used in efficacy tests of drugs and vaccines, none are now suitable for large-scale testing.

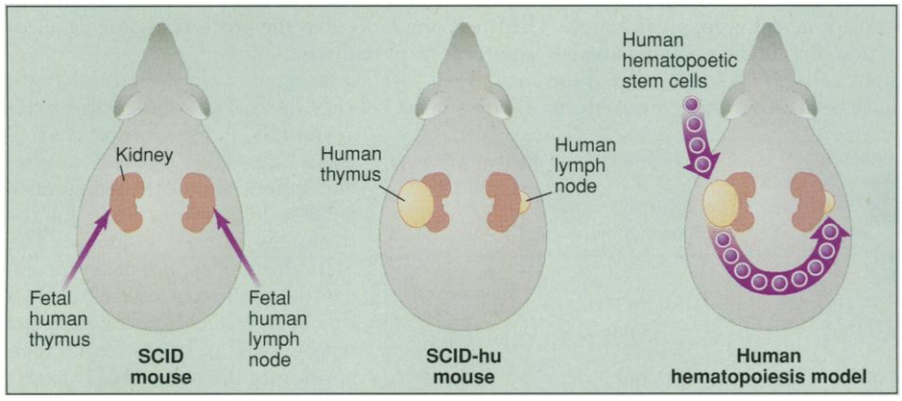

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Fig. 2. Construction of the SCID-hu mouse model. The SCID-hu mouse models human hematopoiesis. It is created by implanting interactive human hematolymphoid organs into the immunodeficient C.B-17 *scid/scid* mouse.

Because no animal model for HIV-1 disease satisfies all of the preclinical needs, the search for alternatives continues. A number of labs are preparing stocks of mice or rabbits, or both, which are doubly transgenic for CD4 and members of the chemokine coreceptor family. These animals may prove to be useful in the preclinical analysis of antiviral compounds and in studying certain aspects of pathogenesis. Perhaps more close at hand are those efforts to optimize rhesus macaque or other nonhuman primate models for infection with HIV-1 or SHIV recombinants. The use of these models is now severely hampered by two limitations: the animal hosts are not inbred (rendering difficult many important immunologic studies) and there are not enough animals available for large-scale testing. Further advances in animal husbandry may remedy these problems and make these models more available for the evaluation of HIV-1–associated pathology, treatment, and vaccines.

Even as animal models for HIV-1 disease are being developed and refined for preclinical testing, persistent efforts should be made to avoid unnecessary animal experiments. This could be achieved, in part, by the development of ex vivo organ-culture systems for studying HIV-1 disease mechanisms. More wide-scale implementation of small, hypothesis-driven human clinical trials is also needed. It is perhaps the latter development that remains most exciting: the best model for human disease is the human with the disease. To the extent that our animal models can approximate that standard, our work with them is likely to be ever more useful.

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