HIV Research in the SCID Mouse: Biosafety Considerations

The SCID (homozygous for severe combined immunodeficiency) mouse engrafted with human cells is a valuable animal model for studies of infection by the human immunodeficiency virus (HIV). Although the guidelines of the National Institutes of Health (NIH) and the Centers for Disease Control (CDC) indicate that biosafety level BSL2 is appropriate for HIV experiments in animals, until now HIV studies in the SCID mouse have been conducted with containment facilities and practices that meet or exceed BSL3.

The National Institute of Allergy and Infectious Diseases (NIAID) sponsored a workshop to review biosafety considerations associated with HIV research in the SCID mouse. Workshop participants included researchers and biosafety specialists and administrators from institutions with SCID mouse facilities, institutions planning SCID mouse facilities, NIH, CDC, and the Food and Drug Administration. A detailed document describing the meeting will be published in the December issue of the *American Society for Microbiology News*.

After a careful review of published and unpublished experimental results, workshop participants agreed unanimously that HIV research in the C.B-17 *scid/scid* mouse does not pose a risk from aerosols and can be conducted safely in BSL2 facilities (a current CDC recommendation) with BSL3 practices. However, BSL2 animal facilities should incorporate design features that prevent escape of HIV-infected animals.

The workshop recommended that studies likely to generate HIV pseudotypes be conducted under BSL3 conditions until evaluation of such potential pseudotypes is possible. *Scid* alleles are being introduced into mouse backgrounds other than C.B-17, and HIV infection of these mice reconstituted with human cells may produce HIV pseudotypes. Furthermore, any research in which HIV is purposely coinfected with mouse amphotropic or xenotropic retroviruses or with human lymphocytotropic viruses such as Epstein-Barr virus and cytomegalovirus may also produce HIV pseudotypes.

There is little to distinguish the biohazards of HIV research in the engrafted SCID mouse from other research with infectious HIV. For all HIV research, careful consideration should be given to minimize risks and to plan procedures that will be used when accidents happen.

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Pseudotypes in HIV-Infected Mice

Paolo Lusso *et al.* (*1*) confirm the well-known fact (*2*) that retroviruses, including the human immunodeficiency virus (HIV), can generate pseudotypes with other enveloped viruses. In particular, they show that a human T leukemic cell line can acquire xenotropic murine leukemia viruses (X-MuLV) after passage through splenectomized, irradiated, and antiasialo–GM1-treated *nu/nu* mice. When the cell line was infected with HIV in vitro, pseudotypes formed between HIV and X-MuLV (*1*). The authors suggest their finding may make data gathered from HIV-infected mice irrelevant.

The critical question is whether and to what extent the possible creation of viral pseudotypes limits the utility of small animal models for HIV. We offer our views based on our work with SCID-hu mice.

Lusso *et al.* "presume" that HIV will directly interreact with X-MuLV within C.B-17 *scid/scid* mice (*3*) that have been either engrafted with human lymphoid organs (SCID-hu) (*4*) or injected with mature human peripheral blood cells (hu-PBL-SCID) (*5*). We have no evidence that this occurs, but the possibility cannot be ruled out. The issue is how frequently it occurs and with what effects. Proof that one can intentionally induce the phenomenon in the laboratory may not be relevant to its significance in vivo.

If such pseudotypes occur in vivo, we do not believe they would compromise the conclusions of our published work. (*6*). We reported that AZT suppressed infection by HIV in the SCID-hu mouse when given 24 hours *before* infection (*6*). The formation of pseudotypes between HIV and X-MuLV should have no impact on the conclusions of this study. Moreover, given the data found in the previous literature (*2*), we assumed that pseudotypes might form in HIV-infected SCID-hu mice and accordingly designed experiments to control for this possibility. The controls have shown that amphotropic pseudotypes did not form in the HIV-infected SCID-hu mouse (that is, mouse spleen cells were not infected (*6*, figure 1); CD4-negative human cells (for instance, in connective tissue) were also uninfected in SCID-hu mice that were viremic with HIV. In other words, pseudotypes did not form between amphotropic or X-MuLV and HIV in SCID-hu mice with a frequency that was high enough to change the interpretation of published experimental data.

Lusso *et al.* suggest that pseudotype formation can account for the absence of certain "characteristic hematologic findings" of human HIV infection in the "SCID-hu/HIV-1 model," namely polyclonal B-lymphocytic activation with hypergammaglobulinemia and CD8+ T-lymphocytic activation. They go on to say that reports of hypogammaglobulinemia in SCID-hu mice may be the result of B cell infection by pseudotyped HIV and that the absence of graft-versus-host (GvH) disease in such animals may result from human T cell suppression by X-MuLV.

The SCID-hu mouse model should not be confused with the hu-PBL-SCID models. These reconstruction approaches are different in design, purpose, and results. Human hematolymphoid organs have been implanted into the SCID-hu mouse in such a way as to circumvent GvH disease (*4*). It has been demonstrated that these mice can be infected with HIV (*7*) and that they can be used to rapidly screen antiviral compounds for efficacy (*6*). It has not been reported that they become hypogammaglobulinemic after infection. It is true that GvH disease has not been initiated by the human T cells within them. These cells were, however, activated by mitogens and by anti-CD3 antibodies when they were removed from the peripheral circulation and tested in vitro (*8*). Our observations suggest that the human T cells have developed selective tolerance during differentiation in the SCID; they are not consistent with generalized viral immunosuppression.

The second SCID model, the "hu-PBL-SCID mouse," was constructed by injection of mature human peripheral blood lymphocytes into SCID mice (*5*). There is no documentation in this system of the development of hypogammaglobulinemia after

HIV infection. T cell suppression was not universally observed either: some (*9*), but not all (*5*), groups have reported GvH disease instead.

Lusso *et al.* urge a "note of caution concerning biosafety measures" as a result of the direct demonstration that pseudotypes can form between X-MuLV and HIV. They suggest that the pseudotypes could be "more pathogenic" than HIV itself. HIV is a formidable pathogen in its own right. Like other retroviruses (including pseudotyped retroviruses), it is not spread by the aerosol route and, at low titer, should be handled under Biosafety Level 2 conditions. Nonetheless, all of our experiments with HIV-infected SCID-hu mice have been conducted under more stringent Biosafety Level 3 (BSL3) precautions. In the case of HIV-transgenic mice (*10*), BSL4 has been used. This level of biocontainment is designed to eliminate the possibility of aerosol spread of HIV. If a pseudotyped HIV were to form, it would also be contained.

Lusso *et al.* show that pseudotypes can form between HIV and X-MuLV. Such phenotypic mixing (between, for example, HIV and HTLV-1, VSV, and herpesviruses) might well be an important component of HIV-induced pathogenesis in humans, and any experiment dealing with HIV in vivo or in vitro should be designed to control for the possibility of pseudotype formation. Yet the body of data already gathered and published about HIV-infected SCID-hu mice speaks for itself. These animals can be used in a safe and productive fashion to study the efficacy of antiviral compounds against HIV in vivo.

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Response: We are grateful to Mc Cune *et al.* for their remarks, which provide an opportunity for a more detailed discussion of some issues raised by our report (*1*) describing the interactions between human immunodeficiency virus type–1 (HIV-1) and xenotropic murine leukemia virus (MuLV). While it is clear that phenotypic mixing is a well-documented interaction between enveloped viruses, our results provided the first evidence that such a phenomenon can involve HIV-1, the causative agent of the acquired immumodeficiency syndrome (AIDS). Similar data have been subsequently reported by other investigators (*2*). The issue of phenotypic mixing has not been previously addressed, to our knowledge, in any publication concerning animal models for HIV infection, including the reports by Mc Cune *et al.* (*3*) and other investigators studying the SCID mouse models.

Although our results may be particularly relevant to the SCID models for HIV infection, our attention was not solely directed to the work of Mc Cune *et al.* We believe the issue of endogenous retroviruses may be of far more general relevance and may also involve other animal models used for the study of human physiology or pathology. The persistent and productive infection with a retrovirus like MuLV may indeed represent an undesired additional variable in the experimental picture. Nonetheless, we have not "suggested" that "data gathered from HIV-infected mice [be] irrelevant." On the contrary, we are thoroughly convinced that the SCID mouse models for HIV infection may provide valuable information for the study of human AIDS.

Mc Cune *et al.* state that *a single* human cell line that we explanted from immunodeficient mice had acquired xenotropic MuLV. However, as clearly documented in table 1 of our paper (*1*), *several* human hematopoietic cell types (six out of six tested) became persistently infected with xenotropic MuLV after heterotransplantation into mice. In addition, although Mc Cune *et al.* focus their attention on the question of "pseudotypes," phenotypic mixing was not the only interaction between HIV-1 and MuLV described in our report: MuLV also dramatically accelerated the time course of HIV-1 expression and cytopathicity in coinfected human T cells (*1*).

We are glad to learn that the preliminary investigation mentioned by Mc Cune *et al.* found no signs of phenotypic mixing between HIV-1 and MuLV in the SCIDhu/HIV-1 model. However, we believe that technical difficulties may hamper the identification of phenotypic mixing in vivo, unless specific experiments are designed to verify this occurrence, and highly sensitive techniques are used. In addition, in their preliminary observations, Mc Cune *et al.* rule out the formation of pseudotypes between HIV-1 and amphotropic MuLV. However, amphotropic MuLV has not been detected in the *Mus* germ line (*4*). Therefore, phenotypic mixing involving amphotropic MuLV is unlikely to occur in laboratory strains of mice.

The question of whether "low-frequency" phenotypic mixing can constitute a problem for the use of murine models for AIDS is difficult to address before the testing of specific therapeutic regimens in vivo. We agree with Mc Cune *et al.* that "the formation of pseudotypes between HIV and X-MuLV should have no impact on the conclusions of [the] study [on the suppressive effect of AZT on HIV-1 infection in SCIDhu mice]." However, in this instance, AZT would be effective against the replicative cycles of both murine retroviruses and HIV-1 (*5*). By contrast, the efficacy of some therapeutic-prophylactic approaches selectively targeted to HIV-1, for example, soluble CD4 therapy, could become questionable.

Concerning the decrease of circulating immunoglobulins in HIV-infected SCID mice, we regret the confusion arising from our report about the various designations used for SCID mouse models engrafted with human cells. This observation was made exclusively for the so-called "hu-PBL-SCID" model (the one described by the group headed by D. Mosier), but not for the "SCIDhu" model (the one described by Mc Cune). Although Mc Cune *et al.* state that these data are still unpublished, they have been repeatedly presented at international meetings and have recently been published (*6*). Unfortunately, this important topic has not been addressed in reports concening HIV-1–infected "SCIDhu" mice (Mc Cune's model).

It is obviously not news to us that "HIV is a formidable pathogen" per se, as our laboratory has contributed significantly to the initial definition of its pathogenic role and routes of transmission. However, the pathogenicity of HIV could become even more formidable if hypothetical variants emerge, making it transmissible by means of nonparenteral routes. This notwithstanding, we stress that we did not attempt to demonstrate or to suggest that phenotypically mixed HIV-1 could be transmitted through

the aerosol route.

We reaffirm that the principle aim of our study was to make researchers aware of the possible interactions between HIV and endogenous retroviruses and, more in general, to the remarkable frequency of infection with xenotropic MuLV observed in human cells heterotransplanted into mice. We believe that such awareness could be important for the correct design and interpretation of some experimental models in vivo.

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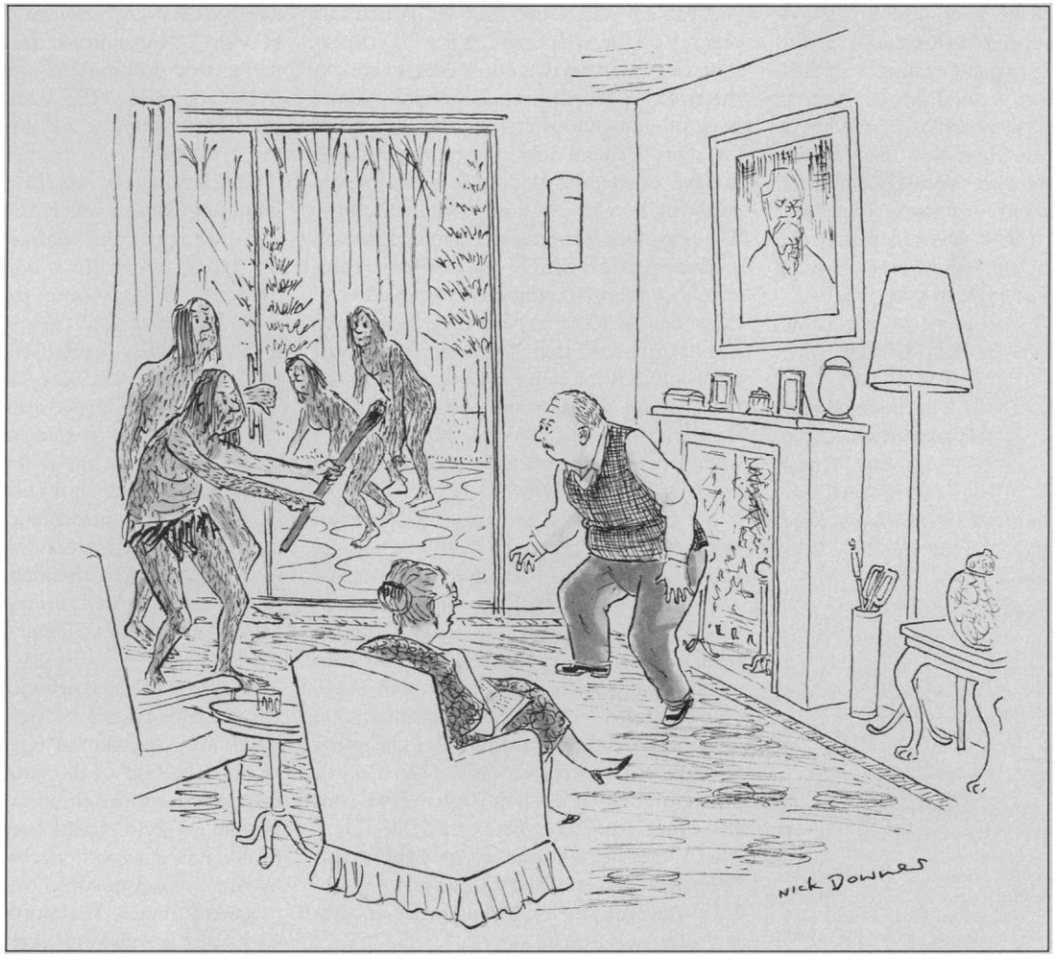

"For heaven's sake George, if it's fire they want, let them take it!"