

## CORRESPONDENCE

# Remarks on the article of Hadas et al.: Transmission of chimeric HIV by mating in conventional mice: prevention by pre-exposure antiretroviral therapy and reduced susceptibility during estrus

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We read with interest the recent paper by Hadas et al., titled “Transmission of chimeric HIV by mating in conventional mice: prevention by pre-exposure antiretroviral therapy and reduced susceptibility during estrus”, first published online on July 25, 2013 (ahead of print) in *Disease Models & Mechanisms* (Hadas et al., 2013). The paper describes an innovative HIV transmission model using conventional mice and the EcoHIV virus [HIV genome with envelope from ecotropic murine leukemia virus (MLV)]. Such a model could offer distinct advantages, as described by the authors: (1) “[this model] provides a simple, small animal platform to investigate interventions to prevent the most frequent route of HIV transmission” and (2) it opens “the extensive repertoire of genetically engineered and mutant mice to study the requirements for [HIV sexual transmission]”. Current models used for studies of HIV sexual transmission have serious limitations: macaque and humanized mouse models are very expensive, limited in availability and might not accurately reflect human HIV transmission events; *in vitro* studies using human tissue explants are limited by poor cell viability and inter-patient variability; and *in vitro* models based on human cell lines usually do not reflect the complexity of natural differentiated genital tract epithelia. An inexpensive murine model for HIV transmission studies would be a welcome addition to the field, but, unfortunately, the new model described in this article also has serious limitations. We cite three major limitations here:

(1) The paper showed that infectious virus was present in reproductive tract tissue (vas deferens) of EcoHIV-infected male mice, but did not demonstrate its presence in semen, nor did it establish that the virus acquired by females co-housed with infected males was sexually transmitted via semen. Transmission could have occurred through biting or exposure to urine or feces. Male mice, including those of the C57BL/6J strain used in this study, are aggressive and have been shown to bite females throughout the estrous cycle (Canastar and Maxson, 2003), as well as following copulation (McGill, 1962). Furthermore, female-to-male and intra-female aggression are not uncommon in mice (Brain, 1999; Morè, 2008) and could facilitate the transmission of virus among group-housed animals. Indeed, a pioneering study on ecotropic MLV (Portis et al., 1987) showed that saliva from infected mice contained high concentrations of infectious virus and that, at least among males, the virus was probably transmitted through biting.

(2) Even if the virus was transmitted via semen, the mechanisms of transmission would be different in mice than humans. One difference is the insemination site. Human semen is deposited in the vagina, whereas, in mice and other rodents, most of the semen enters the uterine cavity (Sobrero and MacLeod, 1962; Zamboni, 1972).

Furthermore, the mouse penis, unlike the human penis, has spines (Rodriguez et al., 2011), which could cause vaginal abrasions during copulation. Finally, the receptor for the ecotropic MLV is a cationic amino acid transporter (CAT-1) that is ubiquitously expressed on almost all murine cells (Hatzoglou et al., 2004), whereas the primary HIV receptor, CD4, and its co-receptors, CCR5 and CXCR4, are expressed primarily on immune cells.

(3) The experiment in this paper that purportedly demonstrated reduced virus susceptibility during estrus was poorly designed. The infection rate of females caged with infected males for one night during estrus was compared with the infection rate of nonsynchronized females caged with infected males for several days. This study can only be meaningful if the infection rate per number of exposures is calculated during each specific phase of the estrous cycle, and other potential routes of exposure are eliminated. A proper experiment studying viral transmission by intercourse on different days of the estrus cycle would be difficult to execute because female mice preferentially mate during estrus, and do not mate at all during diestrus (Fowler and Edwards, 1957).

In our opinion, this article has not established a new mouse model for HIV sexual transmission and, even if subsequent experiments show that the EcoHIV virus can be transmitted via intercourse in conventional mice, the utility of the model for studies on mechanisms of human HIV sexual transmission is severely limited due to differences in viral characteristics between EcoHIV and HIV, and in the events associated with intercourse and reproduction in mice and humans.

### Competing interests

The authors declare no competing financial interests.

### References

- Brain, P. F. (1999). Aggression in female mammals: is it really rare? *Behav. Brain Sci.* **22**, 218.
- Canastar, A. and Maxson, S. C. (2003). Sexual aggression in mice: effects of male strain and of female estrous state. *Behav. Genet.* **33**, 521-528.
- Fowler, R. E. and Edwards, R. G. (1957). Induction of superovulation and pregnancy in mature mice by gonadotrophins. *J. Endocrinol.* **15**, 374-384.
- Hadas, E., Chao, W., He, H., Saini, M., Daley, E., Saifuddin, M., Bentsman, G., Ganz, E., Volsky, D. J. and Potash, M. J. (2013). Transmission of chimeric HIV by mating in conventional mice: prevention by pre-exposure antiretroviral therapy and reduced susceptibility during estrus. *Dis. Model. Mech.* **6**, 1292-1298.
- Hatzoglou, M., Fernandez, J., Yaman, I. and Closs, E. (2004). Regulation of cationic amino acid transport: the story of the CAT-1 transporter. *Annu. Rev. Nutr.* **24**, 377-399.
- McGill, T. E. (1962). Sexual behavior in three inbred strains of mice. *Behaviour* **19**, 341-350.
- Morè, L. (2008). Intra-female aggression in the mouse (*Mus musculus domesticus*) is linked to the estrous cycle regularity but not to ovulation. *Aggress. Behav.* **34**, 46-50.
- Portis, J. L., McAtee, F. J. and Hayes, S. F. (1987). Horizontal transmission of murine retroviruses. *J. Virol.* **61**, 1037-1044.
- Rodriguez, E., Jr, Weiss, D. A., Yang, J. H., Menshenina, J., Ferretti, M., Cunha, T. J., Barcellos, D., Chan, L. Y., Risbridger, G., Cunha, G. R. et al. (2011). New insights on the morphology of adult mouse penis. *Biol. Reprod.* **85**, 1216-1221.

Sobrero, A. J. and MacLeod, J. (1962). The immediate postcoital test. *Fertil. Steril.* **13**, 184-189.

Zamboni, L. (1972) Fertilization in the mouse. In *Biology of Mammalian Fertilization and Implantation* (ed. K. S. Moghissi and E. S. E. Hafez), pp. 213-262. Springfield, IL: Charles C. Thomas.

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## Response to ‘Remarks on the article of Hadas et al.: Transmission of chimeric HIV by mating in conventional mice: prevention by pre-exposure antiretroviral therapy and reduced susceptibility during estrus’

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We recognize the concerns raised in this Correspondence (Andersen and Politch, 2014) regarding the article by Hadas et al. (Hadas et al., 2013) and are responding to alleviate them.

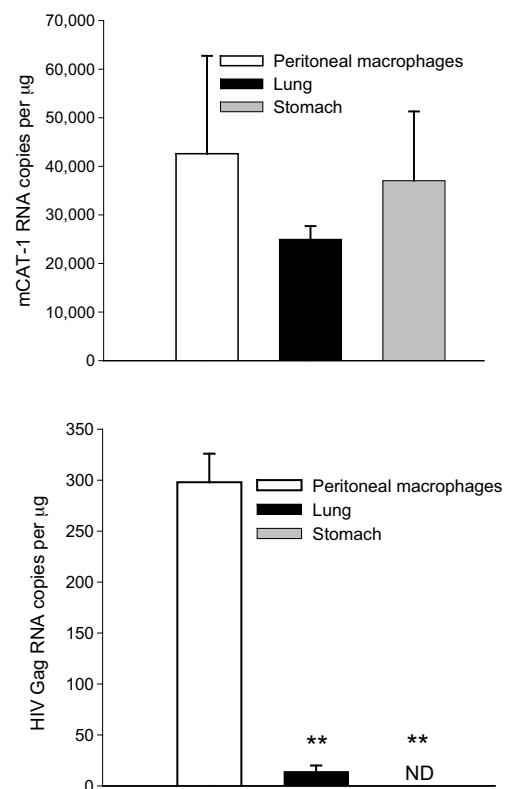
(1) The first issue raised is that EcoHIV-infected male mice might transmit virus to females by biting or other casual contact rather than by mating. This is refuted in principal in a definitive study on the routes of horizontal ecotropic retrovirus transmission in mice (Portis et al., 1987). Portis et al. reported that male-to-female transmission in mice occurred essentially exclusively by mating. They obtained corroborative evidence in finding virus in uterine horns within hours of mating. A virological experiment provided conclusive evidence that vaginal transmission is the only retrovirus transmission route from infected males to females. AKR/J females, although susceptible to WM-E retrovirus infection by injection, were completely resistant to transmission from infected males. This resistance was attributed to viral interference, because females express large amounts of Akv envelope in the reproductive tract and WM-E and Akv belong to the same interference group (McAtee and Portis, 1985). Thus, Portis et al. demonstrated that casual contact between infected males and females during caging together does not transmit retrovirus infection. Studies showing sexual transmission of viruses have followed this definitive paper, echoing the common view that caging infected and uninfected mice results in virus transmission by mating by the male-to-female (Jones et al., 2012; Okada et al., 1998) and female-to-male (François et al., 2013) routes.

As a minor point, in Hadas et al., EcoHIV sexual transmission was not confined to mating infected “aggressive” C57BL/6 males to females as suggested in the Correspondence, but also observed in mating outbred *Foxn1<sup>tm</sup>* males to females (figure 4 in Hadas et al., 2013).

(2) The Correspondence cites biological differences in coitus between humans and rodents. One concern raised is that semen is deposited in the uterus in rodents. Although semen rapidly enters the uterus after insemination in rodents, it is deposited in the vagina, as observed for humans (Carballada and Esponda, 1997; Suarez and Pacey, 2006). The Correspondence notes that the mouse penis is barbed and might cause abrasions to the female reproductive tract during coitus. A detailed histological study of the mouse penis describes “spines whose appearance resembled the filiform papillae on the tongue”, a feature we believe is unlikely to cause abrasions (Murakami, 1987).

The Correspondence suggested that EcoHIV tropism in mice is different than HIV tropism in humans because the murine cellular

receptor for EcoHIV entry, CAT-1, is widely distributed among tissues. EcoHIV tropism to mouse cells is likely to be conferred not only by the cell surface receptor but also by the activity of the viral long terminal repeat (LTR), as is common for retrovirus replication in mice (Celander and Haseltine, 1984); EcoHIV encodes the HIV LTR. To illustrate this EcoHIV tropism here, we show *CAT-1* and HIV *Gag* RNA in tissues from two C57BL/6 mice 10 days after EcoHIV infection (Fig. 1). *CAT-1* is highly expressed in peritoneal



**Fig. 1. Mice were infected with EcoHIV by intraperitoneal injection of  $10^6$  pg HIV p24.** Tissue RNA was isolated and qPCR for HIV *Gag* and glyceraldehyde phosphate dehydrogenase was conducted as described (Hadas et al., 2013). Murine *CAT-1* cDNA was amplified by qPCR according to the manufacturer's instructions using a kit (cat. # 4331182) from Life Technologies, Grand Island, NY, USA. Upper panel: *CAT-1* expression; lower panel: HIV *Gag* expression. ND, not detected. \*\* $P \leq 0.01$  by Student's *t*-test.

macrophages, lung and stomach (upper panel). Although necessary, expression of CAT-1 is insufficient to confer susceptibility to EcoHIV replication. Peritoneal macrophages were productively infected and expressed HIV Gag but neither lung nor stomach was susceptible (Fig. 1, lower panel) ( $P \leq 0.01$ ).

(3) The Correspondence raised a concern that exposure of females in estrus to EcoHIV-infected males for 1 night is not comparable to exposing unsynchronized females to infected males for several nights. For a comparator, see figure 3B in Hadas et al. showing virus burden after 1 night exposure of unsynchronized females, where all placebo-treated females acquired infection (Hadas et al., 2013). We also wish to clarify that mouse mating is not limited to estrus but also occurs in proestrus or metaestrus (Bronson et al., 1968).

All animal models of human processes have shortcomings. Other animal models of HIV sexual transmission in humans employ administration of cell-free virus stock to hormone-treated anesthetized, immobilized females sometimes treated with vaginal irritants and sometimes treated with fire-polished pipettes repeatedly inserted into the vagina. Our demonstration of EcoHIV transmission by coitus in mice can provide a foundation for further model development and prove to be valuable in understanding the primary transmission route of HIV in humans to better control or prevent it.

#### Competing interests

The authors declare no competing financial interests.

#### References

Anderson, D. J. and Politch, J. A. (2014). Remarks on the article of Hadas et al.: Transmission of chimeric HIV by mating in conventional mice: prevention by pre-

exposure antiretroviral therapy and reduced susceptibility during estrus. *Dis. Model. Mech.* **7**, 177-178.

Bronson, F., Dagg, C. and Snell, G. (1968). Reproduction. In *Biology of the Laboratory Mouse* (ed. E. Green). New York, NY: Dover Publications.

Carballada, R. and Esponda, P. (1997). Fate and distribution of seminal plasma proteins in the genital tract of the female rat after natural mating. *J. Reprod. Fertil.* **109**, 325-335.

Celander, D. and Haseltine, W. A. (1984). Tissue-specific transcription preference as a determinant of cell tropism and leukaemogenic potential of murine retroviruses. *Nature* **312**, 159-162.

François, S., Vidick, S., Sarlet, M., Desmecht, D., Drion, P., Stevenson, P. G., Vanderplasschen, A. and Gillet, L. (2013). Illumination of murine gammaherpesvirus-68 cycle reveals a sexual transmission route from females to males in laboratory mice. *PLoS Pathog.* **9**, e1003292.

Hadas, E., Chao, W., He, H., Saini, M., Daley, E., Saifuddin, M., Bentsman, G., Ganz, E., Volsky, D. J. and Potash, M. J. (2013). Transmission of chimeric HIV by mating in conventional mice: prevention by pre-exposure antiretroviral therapy and reduced susceptibility during estrus. *Dis. Model. Mech.* **6**, 1292-1298.

Jones, P. H., Mehta, H. V. and Okeoma, C. M. (2012). A novel role for APOBEC3: susceptibility to sexual transmission of murine acquired immunodeficiency virus (mAIDS) is aggravated in APOBEC3 deficient mice. *Retrovirology* **9**, 50.

McAtee, F. J. and Portis, J. L. (1985). Monoclonal antibodies specific for wild mouse neurotropic retrovirus: detection of comparable levels of virus replication in mouse strains susceptible and resistant to paralytic disease. *J. Virol.* **56**, 1018-1022.

Murakami, R. (1987). A histological study of the development of the penis of wild-type and androgen-insensitive mice. *J. Anat.* **153**, 223-231.

Okada, Y., Abe, E., Komuro, K. and Mizuochi, T. (1998). Heterosexual transmission of a murine AIDS virus. *J. Virol.* **72**, 2541-2543.

Portis, J. L., McAtee, F. J. and Hayes, S. F. (1987). Horizontal transmission of murine retroviruses. *J. Virol.* **61**, 1037-1044.

Suarez, S. S. and Pacey, A. A. (2006). Sperm transport in the female reproductive tract. *Hum. Reprod. Update* **12**, 23-37.

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