Transmission of chimeric HIV by mating in conventional mice: prevention by pre-exposure antiretroviral therapy and reduced susceptibility during estrus

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SUMMARY

Heterosexual transmission accounts for the majority of new human immunodeficiency virus (HIV) cases worldwide. The current approach to investigate HIV heterosexual transmission in animal models involves application of virus stock to the vaginal surface, a method that does not reproduce the physiological conditions of vaginal intercourse that influence the rate of transmission. We have previously described efficient infection of conventional mice using EcoHIV/NL4-3 and EcoHIV/NDK, chimeric HIV molecular clones constructed to express all HIV structural and regulatory genes except envelope, which is replaced by a rodent-tropic envelope gene. Here we investigated whether EcoHIV/NDK-infected male mice transmit virus to females during coitus, and the sensitivity of this transmission to HIV pre-exposure prophylaxis and the estrus state. Our general approach was to allow mating between EcoHIV/NDK-infected male mice and uninfected females for 1-7 nights. At 1-6 weeks after mating, mice were euthanized and virus burdens were measured by quantitative PCR (qPCR) amplification of HIV RNA or DNA in peritoneal macrophages, inguinal lymph node cells, spleen cells or vas deferens, or by ELISA for antibodies to HIV Gag. We found that 70-100% of female mice mated to EcoHIV/NDK-infected males acquired infection. Pericentral treatment of females with either 2',3'-dideoxyctydine (ddC) or tenofovir largely prevented their EcoHIV/NDK infection by mating (P<0.05 and P<0.003, respectively). In males, T cells were dispensable for virus transmission. The rate of EcoHIV/NDK sexual transmission to females in estrus declined sharply (P=0.003) but their infection by injection was unaffected, indicating that the local environment in the female reproductive tract influences susceptibility to HIV. We conclude that this system of EcoHIV/NDK transmission during mouse mating reproduces key features of heterosexual transmission of HIV in humans and can be used to investigate its biology and control.

INTRODUCTION

Since the recognition of acquired immune deficiency syndrome (AIDS), animal models have been developed to study various aspects of human immunodeficiency virus (HIV) infection, pathogenesis and control. The most common route of HIV transmission, sexual transmission (UNAIDS, 2012), has been investigated by simian immunodeficiency virus (SIV) infection of macaques and HIV infection of humanized mice. For infection, virus stock is applied to the female reproductive tract (FRT) or rectum, often after a course of progesterin (Cranage et al., 2008; Denton et al., 2008; Denton et al., 2011; Miller et al., 2005; Sun et al., 2007). These animal models have shown the ability of some antiretrovirals in topical application or injection to prevent or reduce vaginal or rectal virus infection (Cranage et al., 2008; Denton et al., 2008; Denton et al., 2011; Sun et al., 2007). Physiological factors that can influence viral sexual transmission that are absent from these formats are semen as the inoculum, female hormonal cycle changes in susceptibility to infection, and the responses of the FRT to copulation. Semen from HIV-infected men contains cell-free virus and infected cells, and each can mediate infection (Quayle et al., 1997). Cytokines in semen can activate cells of the FRT and the levels of cytokines are higher during acute infection, when HIV sexual transmission is most efficient (Kafka et al., 2012; Lisco et al., 2012; Wawer et al., 2005). Seminal fluid contains enhancers and inhibitors of HIV infection (Münch et al., 2007; Sabatté et al., 2011) that can drive cytokine responses in the FRT that mediate chemotaxis of HIV target cells, possibly amplifying infection (Robertson, 2005). It could be valuable to reproduce these aspects of exposure to HIV that are specific to copulation in model systems.

Our animal model of HIV infection employs EcoHIV, chimeric HIV clones in which the gp120 coding region is replaced by the coding region of the ecotropic murine leukemia virus (MLV) envelope, switching the viral tropism from human to rodent (Potash et al., 2005); all other genes and functions in EcoHIV are derived from HIV. Two viruses have been constructed on Subtype B and Subtype D HIV backbones, EcoHIV/NL4-3 and EcoHIV/NDK, respectively. After inoculation into conventional mice, the viruses infect lymphocytes and macrophages; antiretrovirals, HIV DNA vaccination and anti-EcoHIV CD8+ T cells each can control this virus replication in mice (Hadas et al., 2007; Kelschenbach et al., 2012; Roshorm et al., 2009; Saini et al., 2007).
HIV sexual transmission model

RESOURCE IMPACT

Background
Human immunodeficiency virus (HIV), which causes acquired immunodeficiency syndrome (AIDS), is primarily transmitted via heterosexual intercourse. Constituents of seminal fluid drive cytokine responses in the female reproductive tract that can amplify HIV infection. Moreover, levels of immunity in the female reproductive tract vary over the course of the menstrual cycle and susceptibility to virus infection is influenced by sex hormones, demonstrating the importance of the physiological environment. Although HIV infection and pathogenesis have been modeled fairly extensively in animal models, the currently available models do not allow the dynamic features of viral sexual transmission to be examined. To address this limitation, the present study describes a system for HIV transmission during mating.

RESULTS

To generate a system for viral sexual transmission, chimeric HIV molecular clones (EcoHIV) were constructed and used to infect rodents. The authors demonstrate that EcoHIV-infected male mice efficiently transmit the virus by mating to uninfected females. They show that infection of females can be detected by the presence of antibodies against HIV, or of HIV DNA or RNA in lymphocytes and macrophages. In addition, exposure of females to antiretroviral drugs around the time of mating prevents their infection. Finally, the authors report that female mice in estrus lose susceptibility to EcoHIV sexual transmission yet retain susceptibility to infection via injection.

Implications and future directions
The findings reported here indicate that HIV sexual transmission can be modeled by mating conventional uninfected mice to mice infected with EcoHIV. This novel approach creates the possibility of studying the requirements for sexual transmission of HIV using the extensive repertoire of available genetically engineered and mutant mice while integrating the influences of the male and female mammalian reproductive tissues on infection. Highlighting this potential, the authors demonstrate that the rate of viral transmission declines sharply during estrus, indicating that the local environment in the female reproductive tract influences viral infectivity. Most importantly, the work provides a simple, small animal platform to investigate interventions to prevent the most frequent route of HIV transmission.

Given the ability to investigate HIV replication in conventional mice and the pivotal observation that male to female transmission of retroviruses in mice is almost exclusively through sexual transmission (Portis et al., 1987), this study was conducted to investigate EcoHIV/NMDK sexual transmission and control in mice. We report that female mice acquired EcoHIV/NMDK infection by mating to infected males. This transmission can largely be prevented by pericoital treatment of females with antiretroviral drugs, and transmission efficiency declines during estrus.

RESULTS

HIV is present in human semen and the titer in semen correlates with the efficiency of sexual transmission (Pilcher et al., 2007). To investigate EcoHIV/NMDK expression in male reproductive tissue compared with lymphoid tissue, we evaluated HIV gag RNA burden in the vas deferens and spleen 2 weeks after mouse infection by injection (Fig. 1). HIV RNA was detected at similar levels in both organs; this observation and the previous finding that lymphocytes from EcoHIV/NL4-3-infected mice produce transmissible virus (Potash et al., 2005) indicate that it is likely that the vas deferens can provide infectious virus for sexual transmission.

EcoHIV/NMDK-infected male mice were caged with uninfected female mice for 7 nights prior to tissue collection. C57BL/6 mice were used because they mated more efficiently than other strains in our colony. All mice were euthanized, and HIV gag transcripts and a ubiquitous Y chromosome transcript, to detect male tissue, were measured in peritoneal macrophages (PMs) (Fig. 2). Seven of eight females acquired EcoHIV infection by mating and the uninfected control remained negative (Fig. 2A). Female cell extracts did not contain detectable male RNA, demonstrating that the virus that was detected did not reside in contaminating male cells (Fig. 2B).

Because mice infected by injection of EcoHIV/NL4-3 seroconvert (Potash et al., 2005), detection of anti-HIV antibodies in female mice after sexual transmission can provide additional evidence of infection and viral protein production. Female mice were caged for 1 night with EcoHIV/NMDK-infected males and, after 1 week, PMs were collected to measure virus burden: HIV gag RNA was detected in all females (Fig. 2C). At 6 weeks after mating, plasma was collected from females and anti-HIV/NMDK Gag antibodies were measured by ELISA; plasma from four uninfected mice was also tested (Fig. 2D). The anti-Gag titers were significantly greater (P<0.002) in infected, mated female mice. Thus, female mice can be infected by mating through limited exposure to infected males; they produce EcoHIV/NMDK proteins and mount antiviral responses.

Pre-exposure prophylaxis (PrEP) in human beings can prevent HIV sexual transmission (Celum and Baeten, 2012; Grant et al., 2010) and antiretroviral drugs can prevent EcoHIV/NMDK infection from injection in mice (Hadas et al., 2007). PrEP with 2',3'-dideoxycytidine (dDC) or tenofovir was tested for its efficacy to prevent sexual transmission of EcoHIV/NMDK. Female mice were treated every 12 hours by intraperitoneal (IP) injection of dDC or vehicle, starting 36 hours prior to caging with EcoHIV/NMDK-infected male mice, similar to studies in humanized mice (Denton et al., 2008). Females were exposed to males for 5 nights and were euthanized 1 week after beginning mating. HIV gag burden was measured in PMs (Fig. 3A). EcoHIV was...
transmitted to five out of six vehicle-treated females and to none of five females treated with ddC, indicating that an antiretroviral can prevent EcoHIV transmission by mating \((P<0.05)\). We then limited antiretroviral treatment to two injections and also limited female exposure to infected males to 1 night. Females were treated with tenofovir or vehicle and 12 hours later were housed with EcoHIV/NDK-infected males overnight. The next morning females received another injection and were caged separately from males. At 1 week after exposure to males, females were euthanized and virus burden was measured in PMs (Fig. 3B). Pericoital tenofovir treatment largely prevented EcoHIV infection with statistical significance of \(P<0.003\), analogous to its effect in the prevention of HIV sexual transmission in some humans (Celum and Baeten, 2012; Grant et al., 2010).

With the ability to study HIV sexual transmission through mating infected mice to uninfected mice, we investigated the requirement for T cells by the use of athymic nude (Foxn1nu) male mice, in which T cell development is impaired (Wortis et al., 1971). Nude mice are susceptible to EcoHIV/NDK infection by injection (E.H. and D.J.V., unpublished). EcoHIV/NDK-infected nude males were caged with C57BL/6 females during the nights for 1 week, then females were euthanized to collect PMs and inguinal lymph nodes. Viral RNA was measured in PMs (Fig. 4A) and viral DNA was measured in inguinal lymph nodes to detect an early product of infection (Fig. 4B). All ten mated females carried HIV DNA in lymphocytes and HIV RNA in PMs. We conclude that male mice do not require T cells for sexual transmission of EcoHIV/NDK.

Vaginal susceptibility to HIV in women or to SIV in macaques varies as a result of menstrual cycle or sex hormone administration, with greater susceptibility associated with higher levels of natural or synthetic progesterone; conversely topical administration of estrogen prevents vaginal infection by SIV (Heffron et al., 2012; Smith et al., 2004; Vishwanathan et al., 2011). Female mice can be reliably synchronized in estrus by exposure to male urine (Marsden and Bronson, 1964), permitting evaluation of susceptibility to EcoHIV/NDK infection as it varies with natural hormonal cycling in the FRT. To investigate the effects of estrus on the transmission of EcoHIV/NDK, groups of female mice synchronized in estrus were either caged with EcoHIV/NDK-infected male mice or injected with EcoHIV/NDK stock. Some unsynchronized females were infected by injection for comparison. All mice were euthanized 1 week after virus exposure (Fig. 5). Females synchronized in estrus and unsynchronized females were equally susceptible to EcoHIV/NDK infection by injection (Fig. 5A,B). In sharp contrast, females in estrus were much less susceptible to EcoHIV/NDK sexual transmission than unsynchronized females, with only three of ten mated females infected (Fig. 5C; for comparison see Figs 2-4); however, females in estrus mated efficiently, with nine of ten becoming pregnant. These findings indicate that estrus reduces the susceptibility of female mice to EcoHIV/NDK sexual transmission locally, without systemic effects upon infection. They also provide clear evidence that sexual transmission and not casual contact is responsible for EcoHIV/NDK transmission from infected male to female mice.
The results presented in this work demonstrate that EcoHIV/NDK-infected male mice transmit virus efficiently to female mice by mating. To our knowledge, this is the first description of HIV transmission by coitus in an animal model. The current approaches for experimental study of HIV sexual transmission involve application of concentrated SIV or HIV stocks to vaginal or rectal surfaces in macaques or humanized mice (Cranage et al., 2008; Denton et al., 2008; Denton et al., 2011; Miller et al., 2005; Sun et al., 2007). Both in humanized mice and the mouse mating model described here, virus introduced into the FRT causes systemic infection (Denton et al., 2008; Denton et al., 2011) (Figs 2-4); however, we believe that EcoHIV/NDK transmission by mating can reveal new insights into the physiological conditions in partners of either sex that control the rate and efficiency of HIV sexual transmission. Several points should be made about our findings:

First, HIV transmission by mating in mice was reproducible and highly efficient, with the majority of females showing evidence of infection (Figs 2-4). The efficiency of sexual transmission of EcoHIV/NDK is not surprising; 25 years ago investigators found that sexual transmission was the predominant mode of MLV transmission from infected male to female mice (Portis et al., 1987). Indeed, mating of infected to uninfected mice has been used previously to study transmission of LP-BM5 MLV as a model for HIV sexual transmission (Jones et al., 2012; Okada et al., 1998). Like HIV and murine retroviruses (Jones et al., 2012; Pilcher et al., 2007), EcoHIV/NDK is easily detected in the male reproductive tissue (Fig. 1). The observed HIV or MLV sexual transmission rates in mice were significantly higher than those reported for HIV in humans (Wawer et al., 2005). Although the factors contributing to the higher rate are still being defined, using males at or near the peak of infection and using females outside estrus are likely to play roles (Fig. 5). At the practical level, high rates of EcoHIV/NDK sexual transmission in mice are essential for statistically reliable evaluation of interventions to block this transmission (Fig. 3).

Second, EcoHIV transmission during mouse mating preserves the features of the male and female reproductive tracts, including host factors and cells in seminal fluid that accompany virions from males and those encountered by incoming virus in females. One aspect of mechanisms of HIV sexual transmission can be seen in our use of infected male athymic mice in mating (Fig. 4). These mice lack T cells (Wortis et al., 1971) and, given their ability to transmit virus, our data suggest that T cells are dispensable for transmission of EcoHIV/NDK from males. In this case, the abundant monocytes that are present in the murine male reproductive tract likely contribute virus for sexual transmission (Mullen et al., 2003). Semen contains fragments of prostatic acidic phosphatase that enhance HIV infection (Münch et al., 2007). Soon after HIV infection, semen carries cytokines and β-chemokines that can induce macrophage chemotaxis to the FRT (Kafka et al., 2012), including transforming growth factor β, which is found in semen of uninfected humans and mice (Sharkey et al., 2012a). In tissue culture, semen also induces inflammatory cytokines in human endometrial cells that in turn activate the HIV-1 long terminal.
repeat (LTR), providing a mechanism to locally amplify virus transmission (Kafka et al., 2012). Coitus itself affects the FRT, generating a ‘post-mating inflammatory cascade’ observed in women and female mice, with cytokine and chemokine induction and the migration of macrophages and dendritic cells (Robertson, 2005; Sharkey et al., 2012b). The influx of potential target cells into the FRT might contribute to the observed efficiency of EcoHIV infection. These dynamic features of copulation contributed by both the male and female partners are not reproduced by application of virus stock to the vaginal surface (Miller et al., 2005; Sun et al., 2007). Our goal in establishing the system of chimeric HIV transmission by mouse mating was to preserve these features of copulation for integration of the possible effects of the cells, tissues and responses that affect infection.

Also intrinsic to our model of HIV sexual transmission is the construction of EcoHIV itself; it preserves the entire HIV genome, except gp120. Thus, the functions of or interventions against any of the other HIV proteins or the LTR can be assessed in EcoHIV-infected mice, as exemplified in Fig. 3. This potential utility of this construction is indicated in the observation that SIV reverse transcriptase is relatively resistant to non-nucleoside reverse transcriptase inhibitors (NNRTI) and SIV constructs encoding HIV reverse transcriptase were required to test NNRTI in macaques (Witvrouw et al., 2004). In a similar fashion, EcoHIV constructs encode HIV Vif, which restricts the innate immune effector APOBEC3G to promote HIV infection (Sheehy et al., 2002). An interesting recent study demonstrated that mouse APOBEC3 reduces the sexual transmission of the murine retrovirus swarm LP-BM5, which has been used as a model of HIV (Jones et al., 2012). However, this LP-BM5 does not carry Vif and the antagonism of APOBEC3 and Vif that is present in HIV infection could not be examined.

EcoHIV/NDK sexual transmission was sensitive to antiretrovirals in PrEP (Fig. 3), echoing findings in humans. Oral tenofovir or tenofovir plus emtricitabine prevented HIV transmission between men and within discordant heterosexual couples in some but not all trials (Celum and Baeten, 2012; Grant et al., 2010). In other prevention strategies, vaccination with different HIV DNA constructs has been shown to induce protective immunity and prevent EcoHIV/NL4-3 infection by injection (Im et al., 2011; Roshhoram et al., 2009; Saini et al., 2007). We believe that EcoHIV transmission by mouse mating can complement the existing preclinical screens of HIV prevention through antiretrovirals or vaccination to bring only the most promising approaches to clinical trial.

Finally, the rate of EcoHIV/NDK sexual transmission was greatly reduced in mice in estrus although their overall susceptibility to EcoHIV/NDK infection was not affected (Fig. 5), indicating the importance of the local hormonal environment in the FRT in controlling virus susceptibility. Vaginal transmission of other viruses is under similar control. Mice in estrus are also resistant to vaginal transmission of herpes simplex virus type 2 (Gallichan and Rosenthal, 1996). With peak estradiol levels occurring in mice at estrus and rapidly declining as progesterone levels rise after ovulation (Curtis et al., 1999), our findings are consistent with observations that estrogens tend to prevent and progesterone tends to promote HIV or SIV infection. Topical estrogen can largely prevent vaginal infection by SIV in macaques without affecting their susceptibility to infection by injection (Smith et al., 2004). Macaques are most susceptible to SIV vaginal infection at the peak of progesterone expression and are far less susceptible when progesterone is low (Vishwanathan et al., 2011). Women using injectable hormonal contraceptives containing synthetic progesterone are at enhanced risk for both acquisition and transmission of HIV during intercourse (Heffron et al., 2012). The changes in susceptibility to HIV or SIV vaginal infection have been attributed to changes in local immune effector mechanisms, epithelial wall thickness and pH, among others (Smith et al., 2004; Vishwanathan et al., 2011; Wira and Fahey, 2008). Given the ease of synchronizing mice in estrus, our system could be useful to investigate the contributors to cyclical differences in HIV susceptibility in the FRT. If such differences can be extended to women, they might provide a basis to avoid HIV sexual transmission during the period of greatest susceptibility.

The findings reported here indicate that HIV sexual transmission can be modeled by mating conventional uninfected mice to mice infected with EcoHIV/NDK, opening the extensive repertoire of genetically engineered and mutant mice to study the requirements for this transmission route, integrating the influences of male and female reproductive tissue upon infection, and providing a simple, small animal platform to investigate interventions to prevent the most frequent route of HIV transmission.
MATERIALS AND METHODS

Mice
Male and female C57BL/6 mice and Foxn1nu (nudef) mice from 6-16 weeks of age were either purchased from the Jackson Laboratory (Bar Harbor, ME) or bred in our mouse colony. All use of mice was approved by the St Luke’s-Roosevelt Institutional Animal Care and Use Committee (IACUC). Most experiments reported here employed C57BL/6 mice; the use of nude mice is explicitly noted in the text.

Viruses, infection, real-time PCR and ELISA
EcoHIV/NKD construction, preparation, mouse euthanasia and tissue collection were as described previously (Potash et al., 2005). Plasmid DNA encoding EcoHIV/NKD is available to qualified investigators upon completion of a Material Transfer Agreement. Mouse infection by IP injection of 10⁶ pg HIV p24, ddC or tenofovir administration by IP injection, and real-time PCR amplifying HIV gag DNA or gag or vif RNA, standardized by amplification of gliceraldehyde phosphate dehydrogenase, were as described previously (Hadas et al., 2007). An effective dose of ddC of 200 mg/kg body weight was found previously (Hadas et al., 2007) and tenofovir was administered at 20 mg/kg body weight based on studies of SIV vaginal transmission in macaques (García-Lerma et al., 2010). Every assay of virus burden included cell extracts from uninfected mice as negative controls. The murine Y chromosome transcript was amplified using TaqMan Gene Expression Assay Mm0047710_ml (ABI, Columbia, MD). ELISA to detect antibodies to HIV Gag was as described previously (Saini et al., 2007), except that HIV/NKD p55 was used in solid phase. HIV/NKD p55 was amplified by PCR, cloned into pET-28, expressed in Escherichia coli and purified by nickel-NTA affinity chromatography. HIV burden in groups of mice were compared using Student’s t-test.

Mating
Female mice were caged in groups of five unless otherwise indicated. At 1 week after EcoHIV/NKD infection, individual male mice were housed overnight with two uninfected female mice. Males were removed the next morning and were returned to cages for 1 or more nights as indicated in the text. Synchronization of females in estrus was achieved through the Whitten effect (Marsden and Bronson, 1964; Whitten, 1956): females were housed in pairs in wood shavings from male cages for 1 week prior to housing with infected males. Pregnancy was determined upon necropsy.

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COMPETING INTERESTS
The authors declare that they do not have any competing or financial interests.

AUTHOR CONTRIBUTIONS
E.H., W.C., H.H., E.D. and E.G. conducted experiments; M. Saini and G.B. contributed to tissue collection were as described previously (Potash et al., 2005). ELISA to detect antibodies to HIV Gag was as described previously (Saini et al., 2007), except that HIV/NKD p55 was used in solid phase. HIV/NKD p55 was amplified by PCR, cloned into pET-28, expressed in Escherichia coli and purified by nickel-NTA affinity chromatography. HIV burden in groups of mice were compared using Student’s t-test.

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