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Relationship of menstrual cycle and vaginal infection in female rhesus macaques challenged with repeated, low doses of SIVmac251

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Abstract

Varying susceptibility during menstrual cycling could be a factor for S(H)IV infection risk in female rhesus macaques. We retrospectively determined vaginal SIV infection time points relative to the menstrual cycle in a group of rhesus macaques (n = 11) enrolled in an HIV transmission trial. Eight of nine rhesus macaques became infected around menstruation time.

Keywords

SIV; progesterone; rhesus macaque; menstrual cycle

Introduction

Experimental vaginal S(H)IV infection of rhesus macaques can be more unpredictable than of pigtail macaques [4, 7]. This has limited the use of the species for research on HIV interventions for women. One hypothesis is that susceptibility fluctuations within menstrual cycles and irregular cycles contribute to the unpredictability. To begin to address the issue we here estimated infection time points within the menstrual cycle of rhesus macaques after they had successfully been infected in a recently completed transmission study [1].

A repeat-low dose SIVmac251 virus exposure model was used, thus mimicking the human condition where not every HIV exposure leads to infection. The model was adapted from pigtail macaque models where we have previously summarized infection time points of 43

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SHIV-infected pigtailed serving as controls in HIV prevention research studies [5, 13]. We also documented cycling in 3 additional pigtailed that remained uninfected [5]. We reported that pigtailed macaques have peak susceptibility to SHIV infection one week prior to and one week following menstruation [5]. This is likely due to hormonally-regulated fluctuations in immunity and the vaginal epithelium [14]. Unlike pigtailed, however, wild rhesus macaque groups are seasonal breeders with mating and conceptions during the fall and winter, and births during the spring and summer, along with social hierarchies [3, 8]. Group members' ovulation is reportedly variable, and only some group members give birth, and usually only in spring [3]. It is unclear how the processes play out in captivity, and whether they affect experimental SIV acquisition. In this pilot study, we retrospectively determined menstrual cycling in relationship with SIV transmission time point in 9 infected rhesus macaques, and in 2 macaques that remained uninfected after receiving six vaginal challenges [1].

Methods

Macaques

Twelve captive, cage-housed adult female Indian rhesus macaques (*Macaca mulatta*) of Indian origin served as mock IgG-treated controls during a study testing anti-a4b7 integrin treatment [1]. They were born and housed at the Yerkes National Primate Research Center (YNPRC) of Emory University (Atlanta, GA) [1]. Animals were maintained according to the guidelines of the Institute of Laboratory Animal Resources and with approval of Emory University's IACUC [1, 9].

Virus Challenges, Hormone Analysis

Starting in February, rhesus macaques (n=12) were challenged intra-vaginally with a 1:20 dilution (5,000 TCID₅₀) of SIVmac251 in 1.0 ml of RPMI once weekly, resulting in infection of 10 animals after six or fewer challenges [1]. Challenges began in February and thus happened at the end of cycling season that reportedly lasts until the end of February. To determine the time point when nascent infection reached 50 copies/ml of plasma, viral loads were monitored by using bDNA quantitation on plasma aliquots [1]. Plasma was frozen at -80°C and shipped to the University of Wisconsin National Primate Research Center for progesterone analysis [10, 13]. An infected animal with ID RCd12 [1] had no samples available for analysis. Only 11 of the 12 rhesus macaque plasma samples were available to measure progesterone levels. Rising plasma progesterone was considered evidence of ovulation. We defined cycle day one as the time point post -steepest progesterone decline as previously described [13]. This allowed us to determine the menstrual cycle time point when SIV infection was first detected at greater than 50 copies/ ml of plasma.

Results

Ten animals had progesterone peaks during a total of six virus exposures (Fig. 1), while one animal RIq9 did not (Fig. 1E). All nine infected animals were cycling at time of infection. Their first positive viral load was recorded on menstrual cycle days 1, 6, 6, 8, 8, 12, 12, 14, and 27 (Fig. 2A). Two macaques remained uninfected (Fig. 1E, H); animal RIq9 was not cycling, and cycling status of RTm11 was indeterminate. In Fig. 1, occasional measurements

during winter months preceding the virus challenges are shown to demonstrate that some animals had progesterone peaks, although sampling was too infrequent for meaningful menstrual cycle diagnosis (unconnected dots in Fig. 1). The time point of initial viremia in the nine infected macaques was found to be in the follicular phase (cycle days 1–16) of 8 macaques (95%), as compared with in the luteal phase for only 1 macaque (cycle days 17 – 32, 5%; Fig. 2A). Accounting for an estimated eclipse phase of seven days between virus transmission and first detected plasma viremia, all but one macaque became infected one week prior to or one week following menstruation (Fig. 2B). Progesterone peaks were delayed or stopped fluctuating in March and April for at least one month in all animals except for RIq9 and RTm11 (Fig. 1E, H).

Discussion

We used samples of opportunity to study whether rhesus macaques have fluctuating susceptibility to vaginal infection during menstrual cycling, as we have shown in pigtail macaques. Similar to pigtail macaques, eight of nine infected rhesus macaques preferentially became infected around menstruation time suggesting that rhesus macaques also are more susceptible to SIV following times of high progesterone [3, 8, 14]. This also confirms results by Sodora et al., who found increased virus acquisition in luteal phase when rhesus macaques were exposed in follicular or luteal phase [12]. We were limited to samples from a study in which 9 of 11 females became readily infected. It was not possible to study comprehensively why many female rhesus macaques are reportedly resistant to vaginal infection. All nine infected animals were cycling; it is possible that a lack of cycling in other parts of the year mostly accounts for the resistance. Consistent with this, one of the two resistant animals had no evidence of cycling (Fig. 1E), while our second resistant animal was indeterminate at the time of virus exposure, and could not be sufficiently analyzed because very few blood samples were available (Fig. 1H).

It is possible that other factors contribute to rhesus macaques' resistance to infection. For example, rhesus macaques have considerably thicker vaginal epithelium than pigtail macaques, including greater levels of superficial vaginal keratinization throughout the menstrual cycle, and less thinning during the luteal phase [2, 4, 11].

The exact eclipse phase in each macaque was not known, because it is impossible to determine which of the repeated challenges caused infection. We therefore used seven days for an estimated eclipse phase as in previous studies [6]. It would be beneficial to analyze additional animals after year-round virus inoculations and cycle determinations in frequently collected samples. This could further elucidate the relationship between menstrual cycles and vaginal infection in captive rhesus macaques and further explore the usefulness of repeat low dose mucosal virus exposure in female rhesus macaques.

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References

1. Byrareddy SN, Kallam B, Arthos J, Cicala C, Nawaz F, Hiatt J, Kersh EN, McNicholl JM, Hanson D, Reimann KA, Brameier M, Walter L, Rogers K, Mayne AE, Dunbar P, Villinger T, Little D, Parslow TG, Santangelo PJ, Villinger F, Fauci AS, Ansari AA. Targeting alpha4beta7 integrin reduces mucosal transmission of simian immunodeficiency virus and protects gut-associated lymphoid tissue from infection. *Nature medicine*. 2014; 20:1397–1400.
2. Carias AM, McCoombe S, McRaven M, Anderson M, Galloway N, Vandergrift N, Fought AJ, Lurain J, Duplantis M, Veazey RS, Hope TJ. Defining the interaction of HIV-1 with the mucosal barriers of the female reproductive tract. *Journal of virology*. 2013; 87:11388–11400. [PubMed: 23966398]
3. Gordon TP. Reproductive behavior in the rhesus monkey: social and endocrine variables. 1981; 21:185–195.
4. Hadzic SV, Wang X, Dufour J, Doyle L, Marx PA, Lackner AA, Paulsen DB, Veazey RS. Comparison of the vaginal environment of *Macaca mulatta* and *Macaca nemestrina* throughout the menstrual cycle. *American journal of reproductive immunology*. 2014; 71:322–329. [PubMed: 24521395]
5. Kersh EN, Henning T, Vishwanathan SA, Morris M, Butler K, Adams DR, Guenther P, Srinivasan P, Smith J, Radzio J, Garcia-Lerma JG, Dobard C, Heneine W, McNicholl J. SHIV susceptibility changes during the menstrual cycle of pigtail macaques. *Journal of medical primatology*. 2014; 43:310–316. [PubMed: 24779484]
6. Liu J, Keele BF, Li H, Keating S, Norris PJ, Carville A, Mansfield KG, Tomaras GD, Haynes BF, Kolodkin-Gal D, Letvin NL, Hahn BH, Shaw GM, Barouch DH. Low-dose mucosal simian immunodeficiency virus infection restricts early replication kinetics and transmitted virus variants in rhesus monkeys. *Journal of virology*. 2010; 84:10406–10412. [PubMed: 20686016]
7. McNicholl JM, Henning TC, Vishwanathan SA, Kersh EN. Non-human primate models of hormonal contraception and HIV. *American journal of reproductive immunology*. 2014; 71:513–522. [PubMed: 24716832]
8. Nancy S, Pope MEW, Thomas P. Gordon: The Effect of Season on the Induction of Sexual Behavior by Estradiol in Female Rhesus Monkeys. *Biology of Reproduction*. 1986; 36
9. National Research Council (U.S.). IFLARUS, National Academics Press (U.S.): Guide for the Care and Use of Laboratory Animals. Washington, DC: National Academics Press; 2011. Committee for the Update of Guide for the Care and Use of Laboratory Animals.
10. Saltzman W, Schultz-Darken NJ, Scheffler G, Wegner FH, Abbott DH. Social and reproductive influences on plasma cortisol in female marmoset monkeys. *Physiology & behavior*. 1994; 56:801–810. [PubMed: 7800752]
11. Smith SM, Mefford M, Sodora D, Klase Z, Singh M, Alexander N, Hess D, Marx PA. Topical estrogen protects against SIV vaginal transmission without evidence of systemic effect. *Aids*. 2004; 18:1637–1643. [PubMed: 15280774]
12. Sodora DL, Gettie A, Miller CJ, Marx PA. Vaginal transmission of SIV: assessing infectivity and hormonal influences in macaques inoculated with cell-free and cell-associated viral stocks. *AIDS research and human retroviruses*. 1998; 14(Suppl 1):S119–S123. [PubMed: 9581895]
13. Vishwanathan SA, Guenther PC, Lin CY, Dobard C, Sharma S, Adams DR, Otten RA, Heneine W, Hendry RM, McNicholl JM, Kersh EN. High susceptibility to repeated, low-dose, vaginal SHIV exposure late in the luteal phase of the menstrual cycle of pigtail macaques. *Journal of acquired immune deficiency syndromes*. 2011; 57:261–264. [PubMed: 21546848]
14. Wira CR, Fahey JV. A new strategy to understand how HIV infects women: identification of a window of vulnerability during the menstrual cycle. *Aids*. 2008; 22:1909–1917. [PubMed: 18784454]

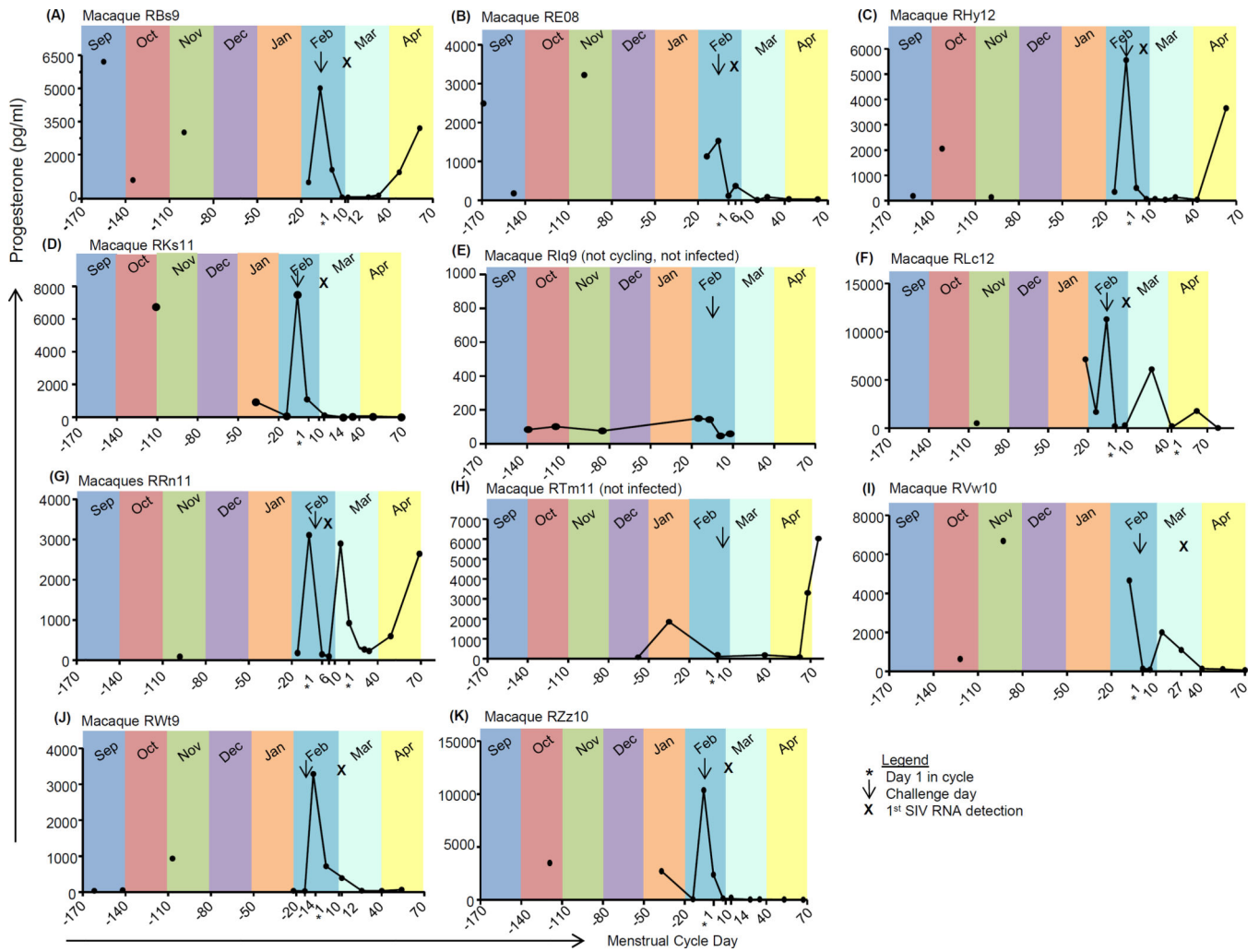


Figure 1.

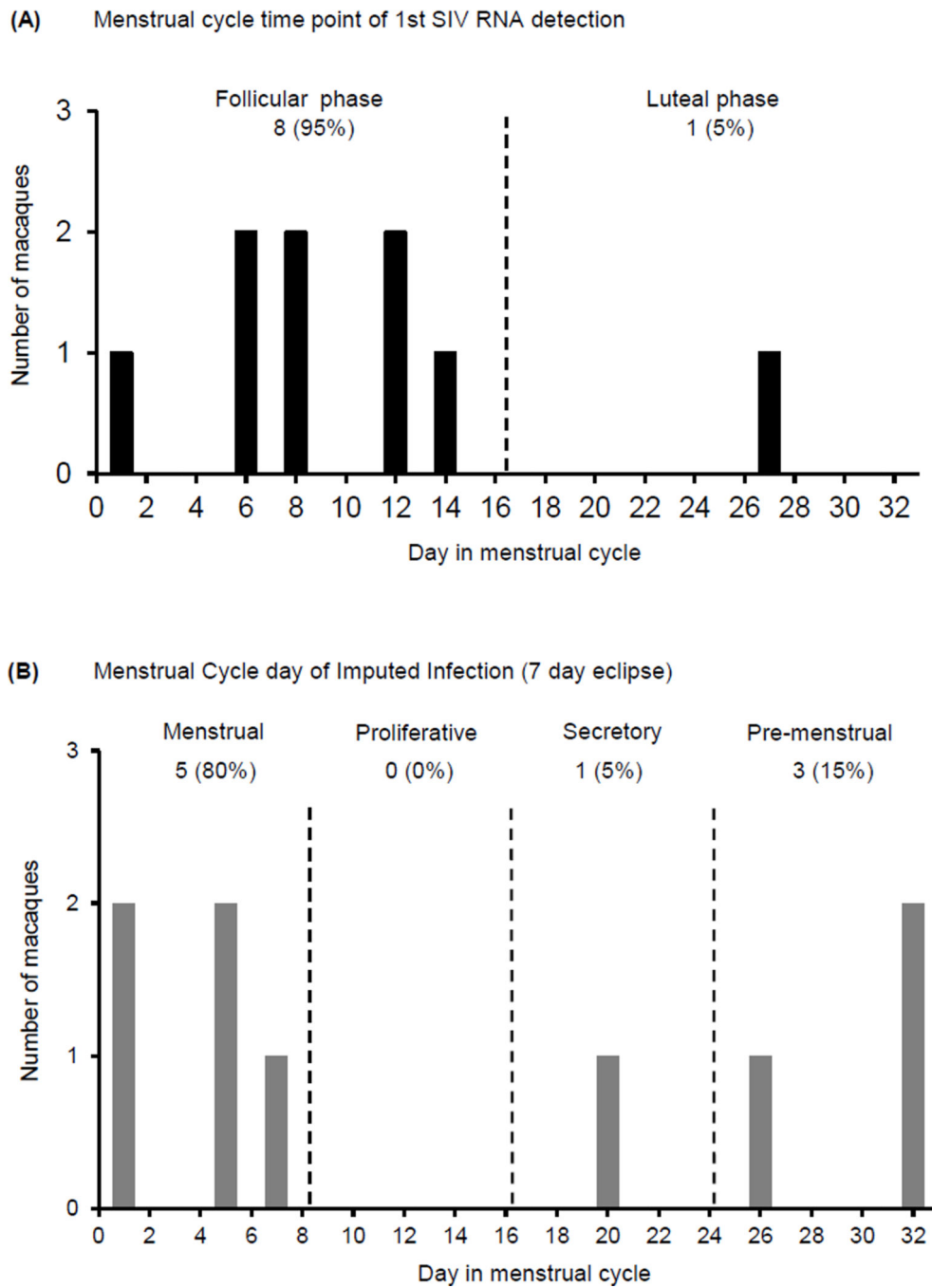


Figure 2.