Published in final edited form as:

J Acquir Immune Defic Syndr. 2016 April 1; 71(4): 353–358. doi:10.1097/QAI.0000000000000890.

Early SIV dissemination after intra-rectal SIVmac251 challenge was associated with proliferating virus-susceptible cells in the colorectum

Yongjun Sui^{1,*}, Eun Mi Lee², Yichuan Wang¹, Alison Hogg¹, Blake Frey¹, David Venzon³, Ranajit Pal², and Jay A. Berzofsky^{1,*}

¹Vaccine Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

²Advanced BioScience Laboratories, Inc. Rockville, MD, 20850

³Biostatistics and Data Management Section, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

Abstract

Objective—Few studies have examined the eclipse time of SIV/HIV infection via the anal route. We aimed to measure the eclipse time after SIVmac251 intra-rectal inoculation, and to investigate the factor(s) associated with early dissemination.

Design—40 macaques were intra-rectally challenged with SIVmac251 three times at 2-week intervals.

Methods—Plasma viral RNA was monitored at 4, 7, 11, 14, 21 and 28 days after infection. Rectal/vaginal tissues were obtained and tissue viral loads (VLs) were measured at day 14 post-infection.

Results—26 out of 40 macaques (65%) had first detectable viral RNAs in the plasma at day 7 after the challenge that led to productive infection. Strikingly, 6 animals (15%) had detectable viral RNA in the plasma as early as at day 4. The Ki67⁺ viral target CD4⁺ T cells in the colorectal tissues were significantly higher in the early or middle-transmitter groups than those in the late-transmitter group. The rectal VL did not correlate with plasma VL at 14-day post-inoculation, but did positively correlate with plasma VLs at days 21 and 28 post-infection.

Conclusions—The median eclipse time after intrarectal challenge was 7 days, with a few early transmitters at 4 days. More rapid viral dissemination was associated with a high frequency of colorectal Ki67⁺CCR5⁺CD4⁺T cells, which fuel the local viral replication. Furthermore, local

*Corresponding authors: Yongjun Sui, Vaccine Branch, National Cancer Institute, National Institutes of Health, 41 Medlars Drive, Bethesda, MD 20892 USA, Ph: 301-435-8350, Fx: 301-402-0549, suiy@mail.nih.gov. Jay A. Berzofsky, Vaccine Branch, National Cancer Institute, National Institutes of Health, 41 Medlars Drive, Bethesda, MD 20892 USA, Ph: 301-496-6874, Fx: 301-480-0681, berzofsj@mail.nih.gov.

Author contribution: Y.S., J.A.B. designed research, interpret the data, and wrote the article.

A.L., R.P. measured the plasma, and tissue VLs.

Y.S., Y.W., A.H., B.F., collected the tissues.

D.V., Y.S. performed the statistical analysis.

Conflicts of interest

There is no conflict of interest.

viral replication in the colorectal tissue during the early stage might affect the plasma VL in a delayed manner. Therefore, to reduce/limit these target cells at the portal of viral entry is essential.

Keywords

eclipse time; intra-rectal inoculation; SIV; viral loads; viral target cells; AIDS virus transmission

Introduction

Following exposure to HIV, viral RNA in the blood is detected only after a lag time, which is referred to as an eclipse time, during which there is a window of opportunity for early interventions [1]. Upon HIV-1 vaginal exposure, the eclipse time is about 10 days in humans, and 7 days in SIV-macaque models [1, 2]. The eclipse time following rectal exposure appeared shorter in some studies due to the more rapid dissemination of SIV [3, 4], while the others showed that the challenge dose affected the eclipse time, with high to low challenge doses leading to 4–8.5 days of eclipse time [5].

An HIV vaccine aiming at achieving sterile protection would need to block or eradicate the virus in the mucosa during or even before the eclipse phase of infection [6]. Despite the importance of rectal infection in the AIDS epidemic, the eclipse time and the factors that affect the colorectal eclipse time have not been fully elucidated. Here we examined the eclipse time of 40 macaques after rectal inoculation of SIVmac251, and investigated the factors that might modulate the eclipse time. Additionally, we measured the early viral loads (VL) in the rectum and vagina, and explored the associations between VLs in these mucosal tissues and plasma. An understanding of these would be helpful in the designing of vaccines to prevent anal HIV transmission.

Methods

Animals, viral challenge, and VL measurements

40 adult Indian rhesus macaques (*Macaca mulatta*) were used with the approval of the National Cancer Institute Animal Care and Use Committee. There were 3 males and 37 females and all were either Mamu-A*01 or A*02 positive, and negative for Mamu-B*08 and B*17. 26 out of total 40 macaques were intracolorectally immunized with a SIV/HIV peptide prime/MVA boost regimen, adjuvanted with TLRs (D-type CpG oligodeoxynucleotide, MALP2, PolyI:C), and recombinant human IL-15. Among them, six macaques received the above-mentioned base vaccine, while the rest of them received base vaccine with B7-DC-Ig (n=6) alone, α -GalCer (n=6) alone, or both (n=8). Six macaques received adjuvants only. The detailed immunization was performed as previously described [7–9]. Eight naive macaques were included. 10 weeks after the last boost, all animals received intra-rectal inoculations of 1:100; 1:100; and 1:50 diluted SIVmac251 stock (provided by Nancy Miller from NIAID, Desrosiers stock-batch number 2/17/2006. Based on the previous animal titration study, one animal ID50 is about 1:500 dilution) sequentially at 2-week intervals until infected. After challenge, plasma SIV RNA levels were measured at day 4, 7, 11, 14, 21, 28 and 2–14 months post-infection. Rectal/vaginal tissue VLs were

measured at day 14 post-infection. 50 copies/ml (or per mg) was the cut-off threshold for detection.

Flow cytometric analysis of viral target cells in the colorectal tissues

7 weeks before the first viral challenge, an open laparotomy was performed on each of the vaccinated and adjuvanted animals to obtain a colorectal wedge. For the naive animals, 10 rectal pinches were collected. The colonic wedge was rinsed with HBSS to remove the mucus, and then cut into 2" pieces. The colonic tissue pieces/rectal pinches were incubated with HBSS containing 5 mM EDTA and 2 mM DTT at room temperature for 15 min twice. The supernatants, which contain intraepithelial lymphocytes (IEL) cells, were collected, pooled and filtered through 100 μ M cell strainers. The collected colorectal IELs were washed and subjected to FACS staining with viability dye and antibody mixtures including anti-CD45 to exclude the dead and CD45 negative epithelial cells. The frequencies of ki67, CD69, CD38 and HLA-DR within CD4⁺ and CD8⁺ T cells were further assessed. About 8000, 6000, and 2000 CD4⁺T cells (mean) were analyzed for vaccinated, adjuvanted and naive animals (Supplementary Fig. 1). The following antibodies were purchased: CD3-PE-Cy7, CD8-APC-Cy7, Ki67-APC, HLA-DR PE-Cy5, and CCR5-PE (from BD Pharmingen); CD69-Alexa Fluor 700 (from Biolegend); CD38-FITC (from STEMCELL Technologies); and CD4-qdot 605 (from eBioscience).

Statistical analyses

We performed statistical analyses with Prism (Graph Pad) and SAS. A two-sided significance level of 0.05 was used for all analyses. The Mann-Whitney or Jonckheere-Terpstra trend tests were used as shown in the figures. Spearman analysis was used for correlations.

Results

More rapid viral dissemination after rectal SIVmac251 exposure was associated with high frequency of colorectal Ki67⁺CCR5⁺CD4⁺T cells

40 macaques were rectally challenged by SIVmac251 at 2-week intervals until infected. Plasma viral RNA was monitored at day 4, 7, 11, and 14 after each challenge, and if the macaques were confirmed infected, they were not challenged further. After three challenges, all the macaques were infected. 26 out of 40 macaques (65%) had first detectable viral RNAs in the blood at day 7 after the challenge that led to the productive infection. Strikingly, 6 animals (15%) had detectable viral RNA in the blood at day 4, whereas another 8 did not show infection until day 11 or 14. When plotting the frequencies of the macaque eclipse times, we found that the middle-transmitters (65%) had a 7-day eclipse time, whereas early-transmitters had an eclipse time of 4 days (15%), and we could define two populations of late-transmitters with 11, and 14 days of eclipse time (Fig. 1a–b). Investigating whether the eclipse time affected the VLs, we found that day14 plasma VL was highest in the early-transmitter group, and then middle >late1>late2-transmitter groups, respectively (Fig. 2a), as predicted. However, there was no significant difference in the set-point plasma VLs (from day 42–147 after infection), or in the rectal and vaginal tissue VLs

at day 14 post-infection among the early, middle, and late-transmitters (Fig 2b–d), suggesting that the eclipse time did not affect these VLs.

Whereas immune activation promotes HIV-1 disease progression [10, 11], a number of studies have suggested that low levels of immune activation at the time of exposure also reduced the risk of HIV-1 infection [12–15]. In our previous study, the level of immune activation in the rectal mucosa, rather than in PBMC, inversely correlated with acute plasma viral loads[7]. However, whether immune activation affects eclipse time is less clear. Before challenge, the macaques had varied levels of CCR5⁺CD4⁺ T cells in the colorectal mucosa. The Ki67⁺ proliferating viral target CCR5⁺CD4⁺ T cells in the colorectal mucosa were significantly higher in the early or middle-transmitter groups than those in the late-transmitter groups, and inversely correlated with eclipse time in both the naive and vaccinated populations, as well as the animals infected after the first inoculation (Fig. 2e–h). This trend was not found in the CD69⁺, or HLA-DR⁺, or CD38⁺ T cells that were CCR5⁺CD4⁺ (data not shown). Thus, the abundance of preexisting Ki67⁺ CCR5⁺CD4⁺ T cells in the rectum/colon predicted rapid viral dissemination. To have a long enough eclipse time for a vaccine to mount a protective recall response, it seems essential to reduce/limit these cells at the portal of viral entry.

Acute rectal VLs correlated with plasma VLs in a delayed manner

After vaginal inoculation, the virus must first establish a small founder population of infected cells at the portal of entry, and then a self-propagating infection in the draining lymph nodes before it is detected in the blood [1]. Upon rectal inoculation, whether similar events are needed is an open question. One difference between the rectum and vagina is that the former has more abundant viral target CD4⁺T cells than the latter. Indeed, both at 14 days after the viral challenge and at the chronic stage, the median rectal VL was over one log higher than the median vaginal VL (Fig. 3a–b). If the virus was first expanded in the rectum before it disseminated to the blood, and the mucosal tissues and blood were well equilibrated, a positive correlation would be expected. However, the day 14 rectal VL did not correlate with blood plasma VL 14 days post-inoculation (Fig. 4a). Instead, day14 rectal VL positively correlated with day 21 and 28, but not day 7 or 14 plasma VLs (Fig. 4b–d). This lag in correlation suggests that initial local viral propagation in the rectal mucosa was important for subsequent dissemination; any interventions that could reduce the rectal VLs at an early time point might affect the plasma VLs at later time points. Moreover, at day 14, an equilibration between virus in rectum and in blood had not been fully established yet. This was in sharp contrast to the scenario at the chronic infection stage, where the correlation between rectal VL and plasma VL was highly significant ($R=0.85$, $P<0.0001$, Fig. 4e). In contrast, the plasma VL did correlate with vaginal VL at day14 (Fig. 4f). At 6 months post-infection, most macaques had undetectable vaginal VL, but those with detectable VL had higher plasma VL ($p=0.011$). The fact that the viral equilibration between colorectal and plasma was delayed whereas that between plasma and vaginal mucosa was not at the acute stage likely reflected the site and route of infection and dissemination, from colorectal mucosa to blood and then to vaginal mucosa. If the animals were infected intravaginally, the reverse pattern might have been seen.

Discussion

To develop an HIV vaccine to prevent anal transmission, information on the eclipse time, and the factors that affect the eclipse time, is desirable. Intra-rectally inoculated SIV in macaque models is the best available model of human rectal transmission of HIV-1 [16]. Small numbers of animals, and high inoculation doses pose a big challenge for studies on eclipse time upon mucosal infections. High dose SIV affected not only the dissemination profile [4], but also the eclipse time [5]. In particular, higher doses of virus resulted in a shorter eclipse time, and more than 10 transmitted/founder viruses [5]. The latter did not recapitulate natural HIV-1 transmission in humans, where typically only one or a few transmitted/founder viruses are found [17]. We addressed this question by using multiple doses of SIVmac251 intra-rectal inoculations. We enumerated the viral variants in the infected animals, and found that out of the 12 animals analyzed, 9 had only 1 variant and 3 had 2 variants [7], suggesting that the challenge was recapitulating human mucosal HIV-1 transmission much more closely than conventional high-dose challenge. Based on the number of variants transmitted, it seems that the dose administered was an intermediate dose [18].

Two potentially confounding factors may complicate the interpretation of this study. One was that 26 out of 40 macaques were vaccinated with an SIV/HIV peptide-prime/MVA-boost vaccine that induced SIV-specific T cell responses. Another was that up to three viral inoculations were given to the animals, which might induce protective viral specific immune responses as well as harmful immune activation, and thus affect the subsequent viral dissemination (10 animals received two, and four animals received three viral inoculations.). However, consistent with no/low antigen-specific antibody responses induced using this regimen[9], we did not observe a difference in viral acquisition time between the vaccinated and the naive animals (Fig. 1a). As the time interval between the last boost and the first viral challenge was 10 weeks, any direct effect of adjuvants on rectal mucosa is negligible. Antigen specific mucosal T cell responses induced by the vaccine and/or prior viral exposure(s) could potentially suppress the viral replication, and thus lengthen the eclipse time. This, however, could be counteracted by the immune activation induced by the prior inoculations. Nevertheless, the distribution of the eclipse time of the vaccinated and the naive animals was similar, suggesting that this regimen of vaccination did not significantly affect the eclipse time (Fig. 1a). Further, the inverse correlation between eclipse time and number of activated CD4⁺ T cells in the mucosa held up even when only the naive animals were examined (Fig. 2g), showing that this correlation did not depend on the vaccination. Moreover, we did not find any association of the number of intrarectal viral challenges with the distribution of the eclipse time (Supplementary Fig. 2), and the distribution of eclipse time of the animals infected after the first inoculation did not significantly differ from that of the animals infected after two or three inoculations, suggesting that the effects of prior viral inoculations (including the possible occult infection) on eclipse time are also minimal (Fig. 1b). Our data showed that the median eclipse time upon intermediate dose anal challenge leading to SIVmac251 infection was about 7 days. An early intervention, ideally, would induce recall responses and block the viral transmission before day 7. However, we did observe viral dissemination as rapid as 4 days in some of the animals. More rapid viral

dissemination in these animals was associated with high frequency of colorectal Ki67⁺CCR5⁺CD4⁺T cells prior to viral inoculation. Studies have demonstrated that high levels of chemoattractant, pro-inflammatory and immune activation genes were induced at the early stage of vaginal [19] or rectal SIV-infection [20], which can recruit and activate virus-susceptible target cells into the mucosa. We found that the preexisting Ki67⁺CCR5⁺CD4⁺T cells in the colorectal mucosa determined the speed of viral dissemination to the periphery. These viral target cells at the portal of viral entry fueled the initial viral replication even before the recruitment of the viral target cells by virus-induced pro-inflammatory chemokines/cytokines, and thus accelerated the viral dissemination. Reducing these cells would be beneficial not only for decreasing the acute VLs as we have observed before [7], but also for extending the eclipse time so that the host has more time to mount protective immunity while the virus is localized to the tissue of entry. The latter is especially important in HIV vaccine development, as more time for the host to induce protective recall responses could make a difference for the ultimate infection outcome. On the other hand, the delayed correlation of rectal VL at Day 14 with plasma VLs at day 21 and 28 suggested that the rectal VL might impact the later plasma VLs. This gave us a hint that any interventions that reduce early rectal VL might still have the chance of reducing systemic VLs. Thus the priority for developing any early interventions would be limitation of viral target cells at the portal of entry combined with the induction of protective immunity at the mucosal sites.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Nancy Miller from the National Institute of Allergy and Infectious Diseases for providing the SIVmac251 challenge stock.

Sources of Funding: Jay Berzofsky is currently receiving NCI intramural funding ZO1 SC 004020, this work was performed under contract awarded to Advanced BioScience Labs, Inc.

References

1. Haase AT. Early events in sexual transmission of HIV and SIV and opportunities for interventions. *Annu Rev Med.* 2011; 62:127–139. [PubMed: 21054171]
2. Miller CJ, Li Q, Abel K, Kim EY, Ma ZM, Wietgreffe S, et al. Propagation and dissemination of infection after vaginal transmission of simian immunodeficiency virus. *J Virol.* 2005; 79:9217–9227. [PubMed: 15994816]
3. Miyake A, Ibuki K, Enose Y, Suzuki H, Horiuchi R, Motohara M, et al. Rapid dissemination of a pathogenic simian/human immunodeficiency virus to systemic organs and active replication in lymphoid tissues following intrarectal infection. *J Gen Virol.* 2006; 87:1311–1320. [PubMed: 16603534]
4. Ribeiro Dos Santos P, Rancez M, Pretet JL, Michel-Salzat A, Messent V, Bogdanova A, et al. Rapid dissemination of SIV follows multisite entry after rectal inoculation. *PLoS One.* 2011; 6:e19493. [PubMed: 21573012]
5. Liu J, Keele BF, Li H, Keating S, Norris PJ, Carville A, et al. Low-dose mucosal simian immunodeficiency virus infection restricts early replication kinetics and transmitted virus variants in rhesus monkeys. *J Virol.* 2010; 84:10406–10412. [PubMed: 20686016]

6. Whitney JB, Hill AL, Sanisetty S, Penaloza-MacMaster P, Liu J, Shetty M, et al. Rapid seeding of the viral reservoir prior to SIV viraemia in rhesus monkeys. *Nature*. 2014; 512:74–77. [PubMed: 25042999]
7. Sui Y, Hogg A, Wang Y, Frey B, Yu H, Xia Z, et al. Vaccine-induced myeloid cell population dampens protective immunity to SIV. *J Clin Invest*. 2014; 124:2538–2549. [PubMed: 24837435]
8. Sui Y, Gagnon S, Dzutsev A, Zhu Q, Yu H, Hogg A, et al. TLR agonists and/or IL-15 adjuvanted mucosal SIV vaccine reduced gut CD4(+) memory T cell loss in SIVmac251-challenged rhesus macaques. *Vaccine*. 2011; 30:59–68. [PubMed: 22041305]
9. Sui Y, Zhu Q, Gagnon S, Dzutsev A, Terabe M, Vaccari M, et al. Innate and adaptive immune correlates of vaccine and adjuvant-induced control of mucosal transmission of SIV in macaques. *Proc Natl Acad Sci U S A*. 2010; 107:9843–9848. [PubMed: 20457926]
10. Giorgi JV, Hultin LE, McKeating JA, Johnson TD, Owens B, Jacobson LP, et al. Shorter survival in advanced human immunodeficiency virus type 1 infection is more closely associated with T lymphocyte activation than with plasma virus burden or virus chemokine coreceptor usage. *J Infect Dis*. 1999; 179:859–870. [PubMed: 10068581]
11. Lawn SD, Butera ST, Folks TM. Contribution of immune activation to the pathogenesis and transmission of human immunodeficiency virus type 1 infection. *Clin Microbiol Rev*. 2001; 14:753–777. table of contents. [PubMed: 11585784]
12. Begaud E, Chartier L, Marechal V, Ipero J, Leal J, Versmisse P, et al. Reduced CD4 T cell activation and in vitro susceptibility to HIV-1 infection in exposed uninfected Central Africans. *Retrovirology*. 2006; 3:35. [PubMed: 16792805]
13. Koning FA, Otto SA, Hazenberg MD, Dekker L, Prins M, Miedema F, et al. Low-level CD4+ T cell activation is associated with low susceptibility to HIV-1 infection. *J Immunol*. 2005; 175:6117–6122. [PubMed: 16237108]
14. McLaren PJ, Ball TB, Wachihi C, Jaoko W, Kelvin DJ, Danesh A, et al. HIV-exposed seronegative commercial sex workers show a quiescent phenotype in the CD4+ T cell compartment and reduced expression of HIV-dependent host factors. *J Infect Dis*. 2010; 202(Suppl 3):S339–344. [PubMed: 20887221]
15. Songok EM, Luo M, Liang B, McLaren P, Kaefer N, Apidi W, et al. Microarray analysis of HIV resistant female sex workers reveal a gene expression signature pattern reminiscent of a lowered immune activation state. *PLoS One*. 2012; 7:e30048. [PubMed: 22291902]
16. Keele BF, Li H, Learn GH, Hraber P, Giorgi EE, Grayson T, et al. Low-dose rectal inoculation of rhesus macaques by SIVsmE660 or SIVmac251 recapitulates human mucosal infection by HIV-1. *J Exp Med*. 2009; 206:1117–1134. [PubMed: 19414559]
17. Keele BF, Giorgi EE, Salazar-Gonzalez JF, Decker JM, Pham KT, Salazar MG, et al. Identification and characterization of transmitted and early founder virus envelopes in primary HIV-1 infection. *Proc Natl Acad Sci U S A*. 2008; 105:7552–7557. [PubMed: 18490657]
18. Varela M, Landskron L, Lai RP, McKinley TJ, Bogers WM, Verschoor EJ, et al. Molecular evolution analysis of the human immunodeficiency virus type 1 envelope in simian/human immunodeficiency virus-infected macaques: implications for challenge dose selection. *J Virol*. 2011; 85:10332–10345. [PubMed: 21795341]
19. Li QS, Estes JD, Schlievert PM, Duan LJ, Brosnahan AJ, Southern PJ, et al. Glycerol monolaurate prevents mucosal SIV transmission. *Nature*. 2009; 458:1034–U1113. [PubMed: 19262509]
20. Lu W, Ma F, Churbanov A, Wan Y, Li Y, Kang G, et al. Virus-host mucosal interactions during early SIV rectal transmission. *Virology*. 2014; 464–465:406–414.

Figure 1.

The distribution of eclipse time after intra-rectal SIVmac251 infection. (a) Immunization status did not affect eclipse time. Day 7 was the median eclipse time in all 40 animals examined (left panel), in the naive controls alone (middle panel), and in all immunized animals excluding the naive controls (right panel). (b) Number of prior viral inoculations did not significantly change the eclipse time. The left panel shows the 26 animals that were infected after the first inoculation; the right panel shows the 14 animals that were infected after the second and third inoculations.

Figure 2.

Eclipse time after intra-rectal SIVmac251 infection inversely correlated with pre-existing Ki67⁺CCR5⁺CD4⁺T cells in the colorectal mucosa. (a–d) Animals with shorter eclipse time had higher day14 plasma VL, but similar levels of day14 rectal, vaginal VLs, and set-point VL (calculated as the average of the geometric means of plasma VLs from day 42 to 147 post challenge). (e–h) Animals with shorter eclipse time had higher frequency of pre-existing Ki67⁺CCR5⁺CD4⁺T cells in the colorectal mucosa in all the animals (e), vaccinated and adjuvanted animals (f), the naive animals only (g), and the animals infected after the first viral inoculation (h). Animals are color-coded: black filled triangles show the immunized animals, orange filled squares show the adjuvant-only treated animals, and red filled circles show the naive animals. Jonckheere-Terpstra tests were used to calculate the p value for trends in a, and e, f, h. Spearman test was used in g.

Figure 3.

The comparisons of VLs in the 2-week (a) and 6-month (b) post-infection plasma and tissue samples.

Figure 4.

Acute rectal VLs correlated with plasma VLs in a delayed manner. (a–d) Day14 rectal VL correlated with Day 21 and Day 28, but not Day 7 or Day 14 plasma VLs. (e) Rectal VL highly correlated with plasma VL at 6 months post-infection. (h) Vaginal tissue VLs at 14 days post-infection were positively associated with concurrent plasma VLs. Animals are color-coded: black triangles show the immunized animals, orange squares show the adjuvant-only treated animals, and red dots show the naive animals.